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THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL
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July, 1957

Pneumococcal Pneumonia Treated with Penicillin and Aspirin.
ROBERT G. PETERSDORF, LEIGHTON E. CLUFF, PAUL D. HOEPRICH, FRANK THOMAS HOPKINS AND WILLIAM P. MCCANN 1

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When BENEMID is used in chronic gout, "...clinical results...have been unequivocally gratifying."[2]

References: 1. Mod. Med. 23:107 (Nov. 15) 1955. 2. J.A.M.A. 154:213 (Jan. 16) 1954.

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PNEUMOCOCCAL PNEUMONIA TREATED WITH PENICILLIN AND ASPIRIN[1]

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In a previous report, the effect of hydrocortisone upon the course of pneumococcal pneumonia treated with penicillin was studied, and it was shown that more rapid defervescence and amelioration of symptoms occurred in hormone-treated patients than in a control group treated with the antibiotic alone (1). Salicylates are both antipyretic and analgesic in action, and in some respects their effects resemble those of adrenal cortical steroids (2).

To determine if acetylsalicylic acid (aspirin) would also afford symptomatic relief to patients with pneumococcal pneumonia treated with penicillin, a controlled study was performed, and the results are presented in this report.

METHOD OF STUDY

Patients. All patients with pneumococcal pneumonia without bacterial complications, (such as meningitis and empyema), admitted to the ward services of the Johns Hopkins Hospital during the period October 1, 1955 to May 31, 1956, were included in the study. Twenty of 87 patients suspected of having pneumococcal pneumonia were eliminated from consideration in the final analysis because of failure to obtain positive bacterial cultures. Therefore, 67 cases were studied in whom bacteriological evidence of pneumococcal infection of the lungs was obtained. Table I summarizes the information on 36 patients treated with aspirin and penicillin, and 31 patients given placebo and penicillin. Although one of the control cases was found to have pneumococcal arthritis of the knee on the day of admission to the hospital, he was included in the analysis. The groups were comparable in age, sex, race and in the incidence of major findings known to influence and indicate the severity of infection or poor prognosis.

Therapy. Each patient was given aqueous potassium penicillin G intramuscularly in a dosage of 300,000 units every 12 hours. This was continued for 7 days or until the patient had been afebrile for 48 hours. Patients were selected at random to receive aspirin or placebo, and neither the physician nor the patient knew which agent was being administered. Aspirin was given in a dosage of 0.6 gram every 4 hours throughout each day (3.6 grams or 12 tablets daily). The same number of placebo (lactose) tablets was given every 4 hours to each patient in the control group. This treatment was continued for 5 days.

Bacteriology. Sputum, pharyngeal and blood culture, in addition to microscopic examina-

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TABLE I

Comparison of Clinical Data on 36 Patients Treated with Aspirin and Penicillin and 31 Patients Treated with Placebo and Penicillin

No. patients	Aspirin		Control		Total	
	No. pts.	%	No. pts.	%	No. pts.	%
	36	100	31	100	67	100
White male	2	6	4	13	6	9
White female	4	11	2	6	6	9
Negro male	20	54	21	68	40	60
Negro female	10	29	4	13	14	22
Under 40 yrs.	19	52	17	54	36	54
40–59 yrs.	11	30	7	23	18	27
60 yrs. or over	6	18	7	23	13	19
Bacteremia	6	17	4	13	10	15
Multilobar disease	5	14	4	13	9	14
Asthma	3	8	0	0	3	4
Previous hist. pneumonia	15	41	15	48	30	45
Pre-existing pul. disease*	6	17	7	22	13	19
Alcoholism	3	8	4	13	7	10
Other disease†	14	38	8	25	22	32

* Asthma, bronchiectasis, chronic bronchitis, emphysema.

† Sarcoidosis, discoid lupus, anemia, arteriosclerotic heart disease, gout, pregnancy, etc.

tion of sputum stained by Gram's method, were done prior to beginning treatment. Serological typing of the pneumococcus was possible in 50 of the 67 cases, and in the remaining 17 cases, the pneumococcus was identified by optochin sensitivity or bile solubility (3, 4).

Other laboratory studies. Hematocrit, erythrocyte sedimentation rate, total and differential leucocyte count, complete urinalysis and x-ray of the chest were done at the time of admission of each patient and repeated frequently during the period of observation. Blood salicylate level was measured on the second day of treatment in 19 patients who received aspirin, and in a similar number of patients who were given placebo. No blood salicylate was found in any of the latter cases.

Clinical observations. Rectal temperature response, time of disappearance of pleuritic pain and other symptoms, and changes in physical findings were carefully noted and recorded for all patients. In addition, 65 patients were studied specifically in regard to subjective manifestations throughout the period of hospitalization; 31 in the control and 34 in the aspirin-treated group. Within a few hours after admission to the hospital and daily thereafter, the patient's symptoms were evaluated independently by two physicians unaware of the therapy being given. The patient was questioned at each interview about sense of well being, cough, chest pain, appetite, tinnitus and vertigo. The severity of symptoms was estimated according to a numerical scale outlined in Table II.

RESULTS

Mortality. There were 2 deaths among the 67 cases of pneumococcal pneumonia, one in the group treated with aspirin and penicillin and one in the group given placebo and penicillin. A 76 year old white man, treated with penicillin and aspirin, had pneumonia, chronic heart failure and emphysema. His tem-

TABLE II

Grading System Used to Evaluate Symptoms in Patients with Pneumococcal Pneumonia

Symptom	Grade				
	0	1+	2+	3+	4+
General Feeling	Normal	Mild malaise and/or weakness	Moderate or severe malaise and weakness	Somnolence; episodes of confusion	Irrational or comatose
Appetite	Normal	Anorexia	Nausea	Nausea and occasional vomiting	Severe vomiting
Cough	None	Occasional	Frequent	Sufficiently frequent to require ℞	Paroxysmal despite ℞
Pain	None	Slight	Severe enough to require ℞	Severe, but alleviated by ℞	Severe, not alleviated by ℞

perature returned promptly to normal, but he became disoriented and had an acute episode of shock and cyanosis following which he died. There seemed to be no relationship between the therapy with salicylates and his death. A 71 year old Negro man had pneumonia and arthritis due to Type 1 pneumococcus and was treated with penicillin and placebo. He was shown to have bacteremia, hyperglobulinemia, and radiolucent areas in the skull and developed episodes of cardiac arrhythmia. It seems likely that he had multiple myeloma, but this was not proven. His lung infection appeared to be under control when he developed a staphylococcal ischiorectal abscess. His unexpected death may have been due to cardiac arrhythmia.

Complications. Suppurative complications such as empyema, meningitis and pericarditis did not occur among the patients in either group, except for the one case in the control group, described above, who was found to have pyarthrosis on the first day of hospitalization.

Pleural effusion was detected radiographically in 2 patients given penicillin and placebo, but in neither case was fluid obtainable by needle aspiration. In each instance the effusion subsided promptly.

Convulsive seizure of the grand mal type occurred in 2 patients for the first time. One of these patients received placebo and had a single convulsion, prior to receiving treatment, without recurrence. No explanation for the seizure, other than the acute infection, was found; however, a history of previous syncopal attacks was obtained. The other patient who received aspirin had a convulsion 11 days after admission to the hospital and 6 days after therapy had been discontinued. This patient also gave a history of having had syncopal attacks, but no cause for the convulsion, other than the infection, was discovered.

Paralytic ileus, severe enough to require gastric aspiration, occurred in 2

patients who received placebo. In each instance the abdominal distention and discomfort were relieved promptly and caused no further difficulty.

Four patients developed *heart failure* during the acute stage of pneumonia. Three of these cases were in placebo group and one was given aspirin. In each instance, the cardiac failure could be attributed to underlying organic heart disease, such as aortic insufficiency and arteriosclerotic vascular disease.

Vascular collapse or shock developed in the 2 fatal cases and in 2 additional patients. A 50 year old Negro male alcoholic developed delirium tremens and transient hypotension on the second hospital day. This patient was leukopenic and blood cultures grew Type 3 pneumococci. The administration of intravenous fluid and sedation with paraldehyde resulted in prompt improvement and the patient recovered completely, although there was delay in resolution of the pneumonia. The second instance of vascular collapse in a patient who received aspirin, may have been attributable to the antipyretic and is presented in detail:

A 60 year old Negro woman (JHH 567772) was admitted to the hospital with cough, fever and right pleuritic pain of 48 hours duration. She had been observed for several years for chronic discoid lupus erythematosus, aortic insufficiency, angina pectoris, mild congestive heart failure, hypochromic anemia and renal insufficiency. There was hyperglobulinemia but "L. E." cells had not been found in the blood. Physical examination revealed a blood pressure of 160/75, temperature 103°F., pulse 100, and respiration 40. There were signs of consolidation over the right lower lung. The lesions of discoid lupus were seen over the face and trunk. The nasal septum was perforated and there were "sabre" shins. A diastolic aortic murmur was heard which was unchanged from that described prior to her hospital admission. *Laboratory examinations showed*: Hematocrit 31.5 per cent, leucocyte count 19,500, and 2+ proteinuria. Blood serological test for syphilis was negative, but 2 treponemal immobilization tests were positive. Sputum, pharyngeal and blood cultures were positive for Type 3 pneumococci. *Hospital course*: The patient was treated with penicillin and aspirin, and her temperature returned to normal within 48 hours. However, on the third day, she became disoriented and tremulous and began to hallucinate. Respirations were Kussmaul in type and on the fifth day she became comatose and hypotensive. Blood salicylate level was found to be 255 microgm./ml. Aspirin was discontinued, and sodium bicarbonate was given intravenously. Improvement was gradual, and during the next 5 days she returned to her previous normal state. The pneumonia cleared without difficulty. With the development of coma the patient's blood NPN rose from 77 mg. per cent to 126 mg. per cent, then returned to 50 mg. per cent during recovery. The plasma CO_2 C. P. was 16 mEq. on admission to the hospital, and did not change appreciably. During convalescence, aspirin was given again for 24 hours without complication.

Comment: This chronically ill woman had Type 3 pneumococcal pneumonia with bacteremia. She was treated with penicillin and aspirin, and on the third day of treatment became disoriented, comatose and hypotensive. Withdrawal of the aspirin was followed by gradual recovery. It is possible that her disturbance of consciousness and vascular collapse were due to salicylate intoxication, exaggerated or precipitated by renal insufficiency.

Significant *hypothermia* developed in a patient given placebo. Rectal temperature fell from 103.8°F. to 94.8°F. during the 12 hours after initiation of treatment, and then returned to normal over the next 12 hours. (Fig. 1). There were no subjective ill-effects.

X-ray of the chest was obtained in 43 patients during and after the fourth week of disease. In 10 cases, (17 per cent of the total of 67), there was *delayed resolution* of the pneumonia beyond 3 weeks; 4 in the aspirin-treated group and 6 in the control group. Complete radiographic clearing finally occurred in all patients except one with bronchiectasis who developed pneumonia again 5 weeks after his first infection. The etiologic organism in 3 of the 10 cases in which resolution was delayed was the Type 3 pneumococcus, although this type was responsible for only 17 per cent of the total cases in the study. Four of the cases of delayed resolution had had bacteremia when first admitted, whereas the incidence of bacteremia in the overall study was only 15 per cent. There was no apparent relationship between delayed resolution and history of previous lung infection.

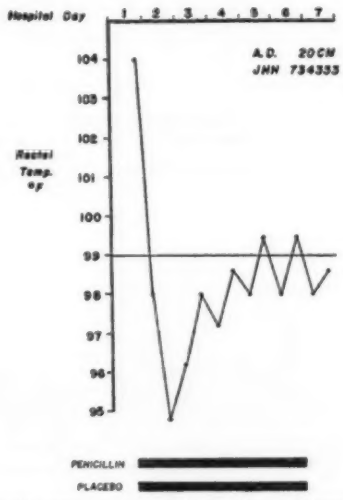

FIG. 1. Rapid fall of body temperature to hypothermic level in a patient treated with penicillin and placebo.

Drug reactions. Aspirin: Nine of the patients in the group treated with aspirin and 4 control patients complained of tinnitus. Nausea or vomiting could not be attributed to the salicylate, as they occurred with the same frequency in both experimental groups. The only significant complications which might have been related to treatment with aspirin have been described above.

Penicillin: Two patients who received aspirin and 2 in the control group developed reactions to penicillin, an incidence of about 6 per cent. One of these reactions was characterized only by fever and is illustrated in Fig. 2. Two other patients developed severe myalgia or arthralgia and fever which disappeared abruptly when penicillin was discontinued (Fig. 2). A fourth probable reaction to penicillin occurred 5 weeks after treatment was discontinued, and was manifested by fever, urticaria and angioedema.

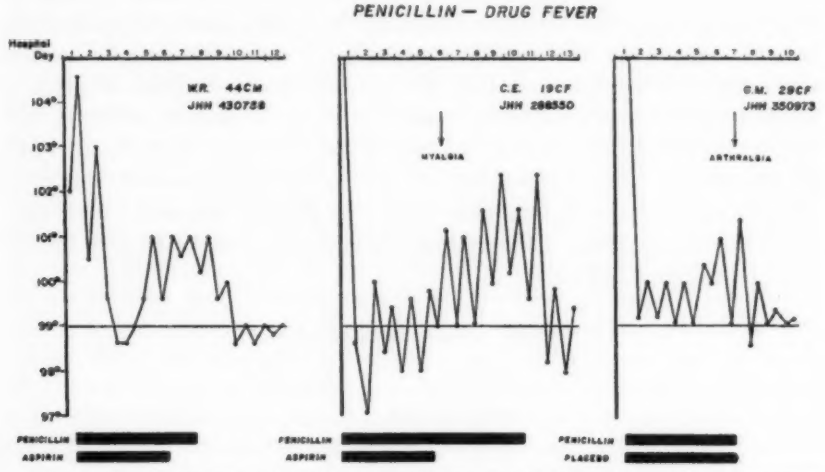

FIG. 2. Recrudescence of fever due to penicillin reaction in patients with pneumococcal pneumonia.

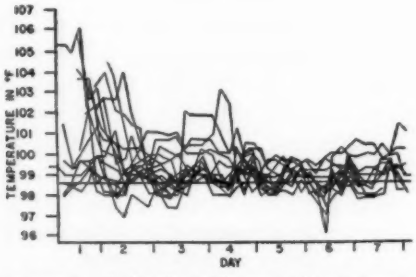

FIG. 3. Temperature curves of the first 15 cases of pneumococcal pneumonia treated with penicillin and aspirin.

Fever. No real difference was noted in the rapidity with which defervescence occurred in the 2 groups. The temperature curves of the first 15 cases in each group are illustrated in Figs. 3 and 4. No "rebound" in fever was seen after withdrawal of aspirin or placebo, and chill did not occur after aspirin or placebo treatment was begun.

Laboratory data. One patient in each group had leucopenia on admission to the hospital, and in both cases, the white cell count rose to normal or higher as the patient recovered. It was possible to type the pneumococcus in 50 of the 67 patients studied, and Type 3 was found in 12 of these cases. The following serological types were also found: Type 1—6 cases, Type 7—6 cases, Type 27—4 cases, Type 19—3 cases, etc. Positive blood cultures were obtained in 10 of the 67 patients; the Type 3 pneumococcus was responsible for 50 per cent, and Type 1 for 20 per cent of the bacteremias.

Bacteriuria. Sixteen patients were specifically studied by Drs. R. Peeler and R. Trever for the presence of pneumococci in the urine. In 6 cases, Gram-

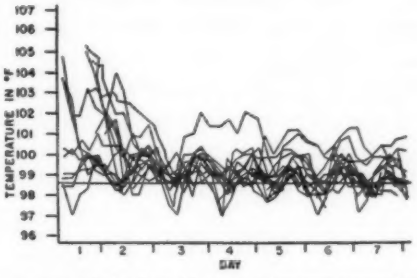

FIG. 4. Temperature curves of the first 15 cases of pneumococcal pneumonia treated with penicillin and placebo.

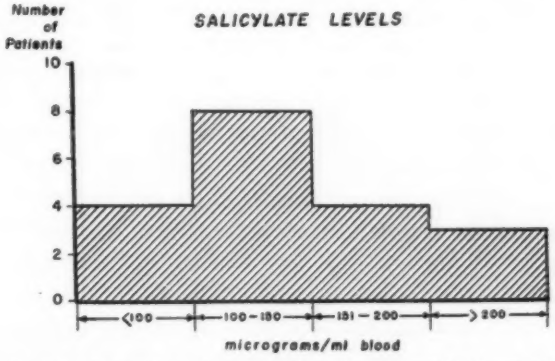

FIG. 5. Blood salicylate levels on the second day of treatment in 19 cases of pneumococcal pneumonia given penicillin and aspirin (0.6 gm. every 4 hours).

TABLE III

Rate of Improvement of Patients with Pneumococcal Pneumonia

Symptom	Treatment Group	Per cent showing improvement			
		Hours after admission			
		24	48	72	>72
General Feeling	A*	88	—	94	100
	P†	68	84	—	100
Appetite	A	64	82	85	100
	P	31	55	69	100
Cough	A	56	62	74	100
	P	47	60	—	100
Pain	A	83	93	—	100
	P	78	89	93	100

* Aspirin.
† Placebo.

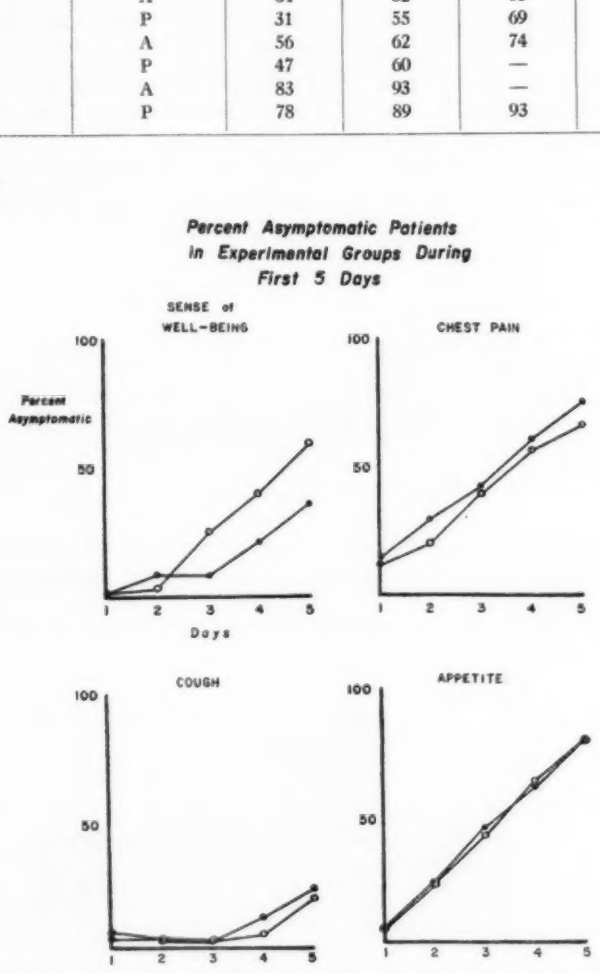

FIG. 6. Rate at which patients with pneumococcal pneumonia became asymptomatic after treatment with penicillin and aspirin (•) or placebo (○).

positive diplococci were seen on smear of the urine collected at the time of the patient's admission. However, in no instance were pneumococci or other Gram-positive cocci grown from the urine of these patients. The presence of diplococci in the urine was not correlated with detectable bacteremia in any of the cases so studied.

Blood salicylate level was determined in 19 cases of the aspirin-treated group and a similar number of patients given placebo. Salicylate was detected in the blood of every patient treated with aspirin in whom a determination was made, while there was none in the control group. The salicylate level in the blood varied from 55 microgm./ml. to 255 microgm./ml. The distribution of the various salicylate levels obtained is illustrated in Fig. 5, which shows that the level in almost half of the cases was between 100 and 150 migrogm./ml.

Subjective response. Of the 65 patients specifically observed for symptomatic response to therapy, 34 received aspirin and 31 the placebo. In general, both groups of patients had symptoms of comparable severity at the time of hospitalization. Table III summarizes the rate of subjective improvement in the groups during the first 3 days of treatment. The small differences, appearing during the first day of treatment, favored the aspirin-treated patient. Analysis of the groups, according to the rate at which the patients became *asymptomatic*, revealed essentially no difference between the cases (Fig. 6).

Aspirin was of no discernible benefit in relieving cough. There were no differences in the severity or duration of pleuritic pain in the groups, and there was no difference in improvement of appetite.

DISCUSSION

A. Effect of aspirin on the patient with pneumococcal pneumonia. Aspirin had little beneficial effect on symptomatic improvement in patients with pneumococcal pneumonia treated with penicillin. Couvy and Popoff (5) treated 140 cases of lobar pneumonia with sodium salicylate before specific antimicrobial drugs were available and reported symptomatic improvement, increased sense of well-being and rapid defervescence in the patients. However, in the cases reported here, the speed with which symptomatic improvement begins in pneumococcal pneumonia treated with penicillin, was not augmented detectably by salicylate. Indeed, the antipyretic effect of salicylate was not observed to influence the rate of defervescence in patients given penicillin.

The "crisis" observed in some patients with untreated pneumococcal pneumonia is accompanied by rapid fall in temperature, occasionally to hypothermic levels (6). The hypothermia which occurred in a patient treated with hydrocortisone, reported in a previous publication (1), was attributed to the antipyretic effect of the adrenal steroid. However, the case observed in the present study, where body temperature fell 10° after institution of penicillin

and placebo treatment, is illustrative of the fact that striking hypothermia may occur even when an antipyretic is not given.

The variation in blood salicylate level in patients on a given dosage of aspirin, is well illustrated by results in this study and has been observed before (7). Although salicylate was detected in the blood of every patient given aspirin, the levels ranged from 55 to 255 microgm./ml., the usual being 100 to 150 microgm./ml. There was no indication that patients with the higher blood salicylate levels were affected differently by the treatment than those with lower blood levels.

The only adverse effect of salicylate observed was in a patient with impaired kidney function. Increased susceptibility to salicylate intoxication has been described in other circumstances associated with renal insufficiency (8). With this one exception, aspirin had no deleterious effect in patients with pneumococcal pneumonia.

B. Manifestations of pneumococcal pneumonia. Cough may not be a troublesome complaint in patients with bacterial pneumonia, but absence of cough is rare (9). However, 3 such cases were observed in this study. Cough occurs with less frequency in aged or debilitated persons or patients with upper lobe pneumonia (9).

Pleuritic pain has been reported in 70 to 80 per cent of patients with pneumococcal pneumonia (9); it was present in 85 per cent of the patients specifically studied in this analysis.

The incidence of *bacteremia* in the present study was 15 per cent, a figure similar to that found in previous studies from this hospital (1). Although the Type 2 pneumococcus is usually stated to be responsible for the largest proportion of positive blood cultures in this disease and Type 3 has been generally a less frequent cause of bacteremia than Types 1 or 2 (10), Type 3 was the causative organism in 50 per cent of the cases with bacteremia. Bacteremia was detected in half of the cases of pneumonia due to this pneumococcal type, a larger proportion than is usually reported (10).

Pneumococcal *bacteriuria* has been reported infrequently, but has occurred in the absence of detectable urinary tract infection (11). In previous reports, bacteriuria had been described as occurring near the time of spontaneous "crisis". Although 6 of 16 patients in this investigation were found to have Gram-positive diplococci in the urine when admitted to the hospital, these bacteria could not be cultured and did not infect mice by the intraperitoneal route. There was no specific relationship of the bacteriuria to detectable bacteremia, and the organisms disappeared from the urine in each case after institution of antibiotic therapy. In one severely ill patient, the pneumococcal etiology of the pulmonary infection was first suspected because of the finding of diplococci in the urine, and was subsequently confirmed by other cultures.

Radiographic clearing of pneumonia is usually complete within 2 weeks

after defervescence, even when specific chemotherapy is not administered (12). Resolution required more than 3 weeks after defervescence in 10 patients in this study. The frequency with which *delayed resolution* followed infection by Type 3 pneumococci, or bacteremia due to any pneumococcal type, is of interest. Previous investigations have shown that delayed resolution is more frequent in Negroes (13). The preponderance of Negro patients in the present study may explain the frequency of this finding.

In adults, *convulsive seizure* can usher in an episode of acute pneumonia (14) and in 2 cases observed here, there were convulsions without a history of previous cerebral dysrhythmia.

No *suppurative complications* developed in the patients after institution of penicillin treatment. *Recurrence of fever* in 4 instances was attributable to penicillin hypersensitivity. These observations bear out the fact that recrudescence of fever in the patient with pneumococcal pneumonia, treated with penicillin, is now more likely to be a drug reaction than a signal of the development of the "classic" suppurative complications such as pericarditis, meningitis or pyarthrosis. The single suppurative complication in this study was present on admission and occurred in a patient who probably had multiple myeloma The frequent association of pneumococcal infection with multiple myeloma has been noted previously (15).

SUMMARY

A controlled study was performed to evaluate the effectiveness of acetylsalicylic acid (aspirin) in alleviating the symptoms of acute pneumococcal pneumonia treated with penicillin. Although patients given aspirin appeared to have experienced more rapid improvement in a sense of well-being, appetite, cough and pleuritic pain during the first 24 hours than placebo-treated controls, there was no detectable difference between the groups after the first day. The rate at which patients in both groups became asymptomatic was the same. The only untoward reaction to aspirin occurred in a patient with chronic renal insufficiency and produced no serious consequences.

The Type 3 pneumococcus was responsible for 50 per cent of the cases of pneumonia associated with detectable bacteremia, and was found in 30 per cent of the patients showing delayed resolution of the lung lesion. Gram positive diplococcal bacteriuria was observed in 6 of 16 cases in whom this examination was made. Recurrence of fever was uniformly attributable to penicillin drug reaction.

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STUDIES ON THE BIOLOGICAL PROPERTIES OF ANTIGEN-ANTIBODY COMPLEXES [1]

I. Anaphylactic Shock Induced by Soluble Antigen-Antibody Complexes in Unsensitized Normal Guinea Pigs. [2]

FREDERICK G. GERMUTH, Jr. and GEORGE E. McKINNON
With the Technical Assistance of Omar Doali and Janet F. Parks
The Department of Pathology, The Johns Hopkins University School of Medicine

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Previous work from this laboratory has indicated that the cardiovascular, renal and granulomatous lymphoid alterations of protracted hypersensitivity develop at a time when antibody forming in the tissues is undergoing rapid combination with excess antigen present in the surrounding fluid and circulating plasma (1). The temporal relationships between the immobilization of antigen by antibody and the production of allergic lesions are consistent with the concept that the tissue alterations produced by a large injection of foreign protein are the result of antigen-antibody combination (1–3).

The precise immunologic mechanisms which lead to alterations in the blood vessels, cardiac valves and renal glomeruli are poorly understood. Since the allergic lesions are initiated while excess antigen is present in the circulation, one wonders how free antibody can get to these sites. It has been suggested that antibody may be carried to these tissues by the circulating leucocytes and that the allergic lesions are produced by the interaction of antibody liberated from these cells with antigen fixed to the tissues (3). However, the tissue alterations of hypersensitivity precede the accumulation of inflammatory cells (1,2). Furthermore, if antibody were transported by the blood leucocytes, it seems likely that such antibody would be quickly neutralized by antigen in the surrounding plasma; for it has been clearly demonstrated that monocytes and lymphocytes readily accumulate antigen from the plasma (4).

In a previous study (1), the possibility was presented that antibody might be transported to the tissues in the presence of excess circulating antigen in the form of soluble antigen-antibody complexes. The formation of soluble antigen-antibody complexes during active antibody production in the presence of excess circulating antigen was suggested by the following considerations. Firstly, it is well known that soluble antigen-antibody complexes are produced *in vitro* when antibody is added to excess antigen (5). Secondly, it was observed

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[2]Presented to the Johns Hopkins Medical Society, January 14, 1957; abstracted in Bulletin of the Johns Hopkins Hospital, April, 1957 and Federation Proceedings, April, 1957.

by one of us (6) that following a single large injection of bovine albumin into rabbits, some of the sera which had been removed at a time when most of the antigen had been immobilized by antibody, underwent spontaneous precipitation on prolonged standing in the ice box. This precipitate was found to be soluble in high concentrations of the specific antigen, which indicated that the precipitate was an antigen-antibody compound. Recently, Sternberger and his colleagues (7–9) have clearly demonstrated the presence of large quantities of soluble antigen-antibody complexes in the sera of animals immunized in a variety of ways and for varying lengths of time with a homogeneous antigen.

The above considerations suggested the possibility that soluble antigen-antibody complexes might play an important role in the production of allergic lesions. The present study indicates that the supernatant fluid of antigen-antibody mixtures prepared with excess antigen to insure the formation of soluble antigen-antibody complexes is capable of eliciting all of the features of anaphylactic shock, when injected into normal unsensitized guinea pigs.

MATERIALS AND METHODS

Antigens: Crystallized bovine plasma albumin (Ba), crystallized egg albumin (Ea) and bovine plasma gamma globulin (BGG) obtained from Armour were dissolved in 0.85 per cent sodium chloride solution and adjusted to pH 7.4 with solid sodium bicarbonate. The protein nitrogen (N) content of the antigen solutions was determined by comparison of the optical density at 280 millimicrons with a standard reference curve of optical density versus antigen nitrogen as determined by the micro-Kjeldahl technique (5).

Antisera: A large series of rabbits were immunized with alum-precipitated antigen according to the schedule employed by Kabat and Mayer (5) or with subcutaneous injections of antigen contained in Freund's adjuvant mixture (10). At appropriate intervals during immunization, their sera were tested for antibody (Ab). For this purpose, samples of blood were obtained from the ear veins and the serum tested for its ability to precipitate large amounts of antigen. Animals with serum containing a high concentration of precipitating antibody were bled by cardiac puncture. Their sera were pooled and kept in a frozen state at $-15°C$ until use.

Preparation of Antigen-Antibody Mixtures: Antigen-antibody mixtures were prepared in a manner such as is routinely employed in the construction of the quantitative precipitin curve; that is, increasing amounts of antigen were added to a series of tubes, each containing 1 ml. of antiserum (5). In the early experiments, the antigen was contained in a volume of 1 ml.; in later experiments, the antigen was added in a volume of 0.2 ml. in order to avoid dilution of the antigen-antibody mixture. The washed precipitates were analyzed in duplicate by the micro-Kjeldahl technique. The supernates were tested for the presence of excess antigen by the addition of 0.1 ml. of potent specific antiserum to 1 ml. of supernate and for excess antibody by the addition of 5 micrograms of the specific antigen nitrogen to 1 ml. of the supernate. By this method of supernate analysis, all of the antigen-antibody systems employed appeared to be immunologically homogeneous; that is, free antigen and free antibody were never found together in the same supernate.

Test for the Capacity of Supernatant Fluid from Antigen-Antibody Mixtures to Induce Anaphylactic Shock: The clear supernatant fluids obtained by centrifugation of antigen-

antibody mixtures prepared throughout the range of the precipitin curve were injected into the hind leg vein of normal male guinea pigs weighing 250–350 grams. The volume injected was usually 2 ml. In order to rule out anaphylactoid phenomena which are occasionally produced by certain sera, an equal quantity of whole antiserum was injected into several animals in each experiment. None of the antisera used exhibited primary toxicity. Amounts of antigen far in excess of those used in the present experiment produced no reactions in normal guinea pigs.

But for minor exceptions, guinea pigs injected with supernates from antigen-antibody mixtures containing *excess antibody* showed no signs which could be interpreted as those of anaphylactic shock. However, many of the normal guinea pigs injected with supernatant fluids from antigen-antibody mixtures prepared with *excess antigen* exhibited all of the features characteristic of anaphylactic shock. Within 1 minute or less after the injection of the supernatant fluid, some of the guinea pigs exhibited marked restlessness, sneezing, coughing, scratching of the nose and mouth, the passage of feces and urine, and rapid and irregular respirations. The reaction was graded slight or moderate depending upon the degree of respiratory embarrassment. Other animals rapidly developed spasmodic sneezing and jumping followed by convulsions. Those that survived were considered as having a pronounced reaction; however, most of the animals showing these signs died within 1 to 5 minutes after the injection of the supernatant fluid. Occasionally, instead of showing a prompt reaction, the animals exhibited delayed respiratory difficulties and marked weakness similar to the signs observed by Anderson (11) and by Weil (12) in poorly sensitized animals and in fully sensitized animals when the challenging injection of antigen was administered by a route which favored slow absorption. Some of these animals died 3 to 24 hours after injection. All of the animals which died were autopsied as soon as possible and sections were taken of the lungs for microscopic examination.

EXPERIMENTAL RESULTS

A. The Production of Anaphylactic Shock by Mixtures of Antibody and Excess Antigen

Experiment 1: The Anaphylactigenic Properties of Supernates from Certain Mixtures of Crystallized Egg Albumin (Ea) and Anti-Egg Albumin Rabbit Serum I.

The antiserum was obtained from rabbits selected for their high antibody titer after immunization with alum-precipitated Ea for 1 month. To tubes containing 1 ml. of antiserum was added 1 ml. of antigen solution containing varying amounts of Ea. This procedure was carried out in the cold with cold reagents. After 48 hours at 3–5°C, the tubes were centrifuged at 2200 r.p.m. for 40 minutes in a refrigerated centrifuge. The supernatant fluid from each set of tubes prepared with identical amounts of antigen was pooled and injected in 2 ml. volumes into normal guinea pigs.

The results are outlined in Table I and presented diagrammatically in Figure 1. None of the 12 animals receiving whole antiserum or supernatant fluid from antigen-antibody mixtures containing *excess antibody* exhibited ill effects. However, 12 of 14 guinea pigs receiving comparable volumes of supernatant fluid from antigen-antibody mixtures prepared with *excess antigen* showed

TABLE I

The Effect in Guinea Pigs of the Intravenous Injection of Supernatant Fluid from Antigen-Antibody Mixtures Obtained by the Addition of Varying Amounts of Crystallized Egg Albumin to 1 ml. of Specific Antiserum I[1]

No. of Guinea Pigs	Amount of EaN[2] added to 1 ml. of antiserum (794 micrograms AbN), micrograms.	Total Vol. of supernate injected, ml.	Supernate positive for[3]	No. of Guinea Pigs Showing:			
				No effect	Mod. Shock	Severe Shock	Death
4	0	2.0	Ab	4	0	0	0
4	40	2.0	Ab	4	0	0	0
4	90	2.0	trace Ab	4	0	0	0
Total = 12			Ab	12	0	0	0
10	180	2.0	At	1	2	4	3
4	240	2.0	At	1	0	0	3*
Total = 14			At	2	2	4	6

[1] Obtained from rabbits immunized for 1 month with alum-precipitated Ea. 1 ml. antiserum + 1 ml. of antigen solution gave following precipitate values:

EaN added, micrograms	Precipitate N, micrograms	Supernate + for
21	323	Ab
42	515	Ab
63	749	Ab
84	829	Ab trace
93	887	At trace (AbN/ml. = 794 micrograms)
120	840	At
180	649	At
240	457	At

[2] Antigen contained in 1 ml. saline.

[3] Ab = antibody; At = antigen.

* One death immediately; other 2 within 24 hours.

signs characteristic of anaphylactic shock; 4 of these died within 5 minutes; 2 others died within 24 hours. At autopsy, the animals which died rapidly showed marked pulmonary emphysema and at times, small pulmonary hemorrhages. Microscopic examination revealed, in addition to dilated alveoli, what appeared to be contracted bronchioles and peribronchial edema. No capillary thrombi were observed. Animals showing delayed death showed no remarkable abnormalities.

Experiment 2: The Anaphylactigenic Properties of Supernates from Certain Mixtures of Crystallized Egg Albumin (Ea) and Anti-Egg Albumin Rabbit Serum II.

The antiserum was procured from animals receiving alum-precipitated Ea for 1 month. The supernates were obtained in the same manner as in Experiment 1. All 8 animals receiving supernates from Ea-anti Ea serum containing

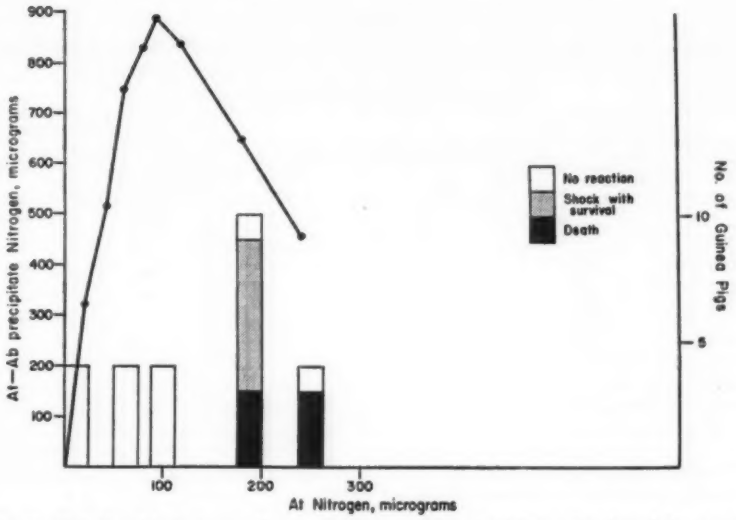

FIG. 1. Relationship between the precipitin curve (Ea-anti Ea system) and the capacity of supernatant fluid to produce shock. (After Table I)

excess antibody showed no untoward reaction whereas 19 of 26 guinea pigs receiving supernates prepared from mixtures of Ea and antiserum containing *excess Ea* showed typical anaphylactic reactions. Seven of these 19 animals died within 6 minutes after injection of the supernates.

The data of this experiment are summarized in Table II and in Figure 2.

Experiment 3: The Anaphylactigenic Properties of Supernates from Certain Mixtures of Crystallized Bovine Albumin and Anti-Bovine Albumin Rabbit Serum R.

The antiserum was obtained from rabbits developing high serum antibody concentrations following immunization with alum-precipitated crystallized bovine albumin for 1 month. Antigen-antibody mixtures were prepared as in Experiment 1, except that the antigen was contained 0.2 ml. of saline rather than 1 ml.

The results are recorded in Table III. One of 9 animals receiving supernates from antigen-antibody mixtures prepared in *antibody excess* exhibited symptoms of moderate shock; the other 8 showed no reaction whereas 14 of 16 animals injected with supernates from antigen-antibody mixtures prepared with excess antigen showed definite anaphylactic reactions and 4 of these died in acute anaphylactic shock. Autopsy of the animals dying in shock revealed marked pulmonary emphysema.

Experiment 4: Anaphylactigenic Properties of Supernates from Certain Mixtures of Crystallized Bovine Albumin and Anti-Bovine Albumin Rabbit Serum F_1.

TABLE II

The Effect in Guinea Pigs of the Intravenous Injection of Supernatant Fluid from Antigen-Antibody Mixtures Obtained by the Addition of Increasing Amounts of Crystallized Egg Albumin to 1 ml. of Specific Antiserum II[1]

No. of Guinea Pigs	Amount of EaN[2] Added to 1 ml. Antiserum (533 Micrograms AbN/ml.), Micrograms	Vol. of Supernatant Fluid Injected, ml.	Supernate Positive for[3]	No. of Guinea Pigs Showing:			
				No Effect	Mod. Shock	Severe Shock	Death
8	50	2	Ab	8	0	0	0
5	100	2	At trace	3	1	1	0
3	150	2	At	1	1	0	1
10	200	2	At	2	2	3	3
4	400	2	At	1	1	1	1
4	800	2	At	0	1	1	2
Total							
8			Ab	8	0	0	0
26			At	7	6	6	7

[1] Obtained from rabbits immunized for 1 month with alum precipitated Ea. 1 ml. antiserum + 1 ml. of antigen solution gave following precipitate values:

EaN added, micrograms	Precipitate N, micrograms	Supernate Positive for:
50	509	Ab
75	608	0 (AbN/ml. = 533 micrograms)
100	580	At trace
150	443	At
400	164	At
800	87	At

[2] Antigen contained in 1 ml. of saline.

[3] Ab = antibody; At = antigen.

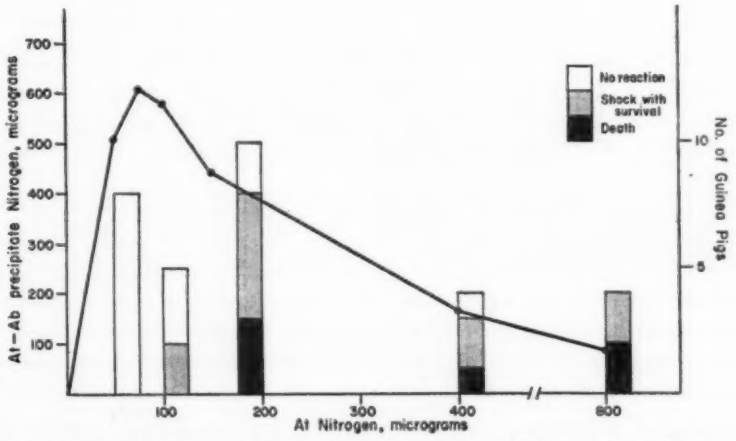

FIG. 2. Relationship between the precipitin curve (Ea-anti Ea system) and the capacity of supernatant fluid to produce shock. (After Table II)

TABLE III

The Effect in Guinea Pigs of the Intravenous Injection of Supernatant Fluid from Antigen-Antibody Mixtures Obtained by the Addition of Varying Amounts of Crystallized Bovine Albumin to 1 ml. of Specific Antiserum R_1[1]

No. Guinea Pigs	Amount of BaN Added to 1 ml. Antiserum (750 μg AbN/ml.); Micrograms[2]	Vol. of Supernatant Fluid Injected, ml.	Supernate Positive for:	No. of Guinea Pigs Showing				
				No Effect	Slight Shock	Mod. Shock	Severe Shock	Death
5	53	2	Ab	4	0	1	0	0
4	105	2	Ab	4	0	0	0	0
7	210	2	At	1	2	0	2	2
9	420	2	At	1	5	1	0	2
Total = 9			Ab	8	0	1	0	0
Total = 16			At	2	7	1	2	4

[1] Antiserum obtained from rabbits immunized with alum precipitated crystallized bovine albumin for 1 month; antibody nitrogen content approx. 750 microgms./ml.; equivalence point of At = 126 microgms AtN.

[2] Antigen contained in 0.2 ml. of saline.

The procedure used in this experiment was similar to that employed in Experiment 3. The data are presented in Table IV and Figure 3. Again, only guinea pigs injected with supernates prepared from mixtures of antiserum and *excess antigen* showed symptoms characteristic of anaphylactic shock.

Experiment 5: Variations in the Shocking Capacity of Supernates Obtained from Antigen-Antibody Mixtures Prepared with Excess Antigen.

In numerous exploratory experiments in which various antisera were employed to determine the capacity of antibody-excess antigen mixtures to shock normal guinea pigs, great variation in degree of shock was encountered. It was not clear whether this variation was due to differences in the quality of antibody in the antisera[3] to slight differences in the conditions under which the antibody-excess antigen mixtures were prepared[4] or to individual differences in the susceptibility of normal guinea pigs to the shocking action of the supernates. In order to determine whether the method of preparation of antibody-excess antigen mixtures might influence the anaphylactigenic capacity of the supernatant fluid, antibody-excess antigen mixtures were made under a variety of conditions with a single antiserum. The results are recorded in Table V. Although there were marked variations in the degree of shock produced in guinea pigs injected with supernates prepared on different occasions, there was no clear-cut relationship between the anaphylactigenic capacity of the

[3] Quality of antibody refers to the differences in reactivity of the spectrum of antibodies to a homogeneous antigen in precipitin, complement-fixation and allergic reactions.

[4] It is well known that the excess antigen portion of the precipitin curve is more difficult to reproduce than the excess antibody portion. Variations in the rate at which excess antigen is added to antibody may markedly influence the amount of precipitation obtained after the usual incubation period of 48 hours in the cold.

TABLE IV

The Effect in Guinea Pigs of the Intravenous Injection of Supernatant Fluid From Antigen-Antibody Mixtures Obtained by the Addition of Varying Amounts of Crystallized Bovine Albumin to 1 ml. of Specific Antiserum F_1[1]

No. of Guinea Pigs	Amount of BaN Added to 1 ml. Antiserum (797 μg AbN/ml.); Micrograms[2]	Vol. of Supernatant Fluid Injected, ml.	Supernate Positive for:	No. of Guinea Pigs Showing:				
				No Effect	Slight Shock	Mod. Shock	Severe Shock	Death
4	0	2	Ab	4	0	0	0	0
10	70	2	Ab	10	0	0	0	0
8	200	2	At	1	3	3	0	1
10	410	2	At	0	2	1	0	7
Total = 14			Ab	14	0	0	0	0
Total = 18			At	1	5	4	0	8

[1] Antiserum obtained from rabbits receiving 1 injection per week for a total of 19 weeks of 20 mg. bovine albumin in Freund's adjuvant; antiserum was diluted 1:2.8 with saline for use in the above experiment. The resulting solution contained 797 microgms. AbN/ml. 1 ml. of this antiserum and 1 ml. of antigen dilution gave the following precipitate values:

BaN Added, Micrograms	Precipitate N, Micrograms	Supernate + for
82	781	Ab
101	862	Ab
136	930	0
152	949	Trace At (ABN/ml. = 797 micrograms)
200	862	At
410	53	At

[2] Antigen contained in 0.2 ml. saline.

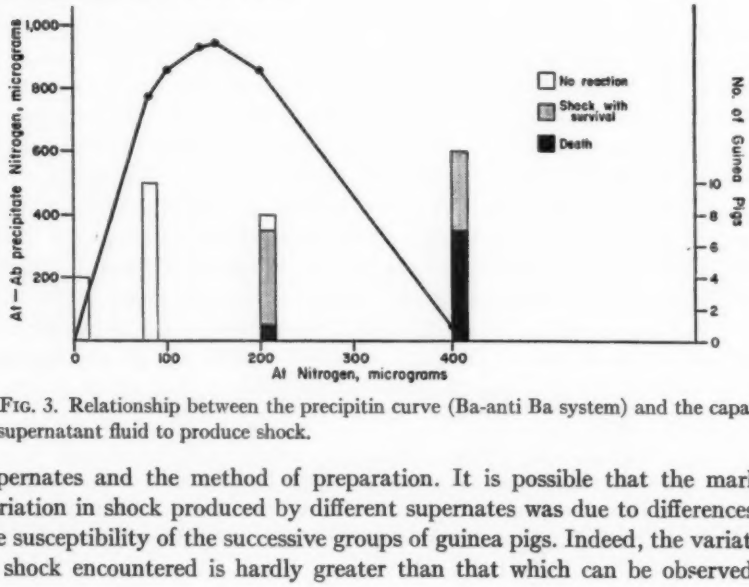

FIG. 3. Relationship between the precipitin curve (Ba-anti Ba system) and the capacity of supernatant fluid to produce shock.

supernates and the method of preparation. It is possible that the marked variation in shock produced by different supernates was due to differences in the susceptibility of the successive groups of guinea pigs. Indeed, the variation in shock encountered is hardly greater than that which can be observed in

TABLE V

Variations in Shocking Capacity of Supernatant Fluid Obtained from Antigen-Antibody Mixtures Produced in Antigen Excess

No. of Guinea Pigs	Method of Preparation of Antigen-Antibody Mixtures[1]	No. of Guinea Pigs Showing:				
		No Effect	Slight Shock	Mod. Shock	Severe Shock	Deaths
8	Instantaneous addition of At to antiserum; cold reagents; incubated at 0–5°C for 24 hrs.	0	6	2	0	0
9	Instantaneous addition; cold reagents; incubated at room temperature for 1 hr.	1	3	1	3	1
10	Instantaneous addition; cold reagents; incubated at 0–5°C for 24 hrs.	7	1	1	1	0
6	Instantaneous; cold reagents; incubated at 0–5°C for 24 hrs.	2	2	1	0	1
7	Instantaneous; warm reagents; incubated at 37°C for ½ hr.; placed in ice box overnight.	0	4	1	1	1
Total = 40	Instantaneous addition of At to antiserum.	10	16	6	5	3
10	Slow addition of At to antiserum (20 sec); incubated for 1 hr. at room temperature.	0	0	4	2	4
10	Slow addition (20 sec); incubated at 0–5°C for 24 hrs.	3	5	2	0	0
15	Slow addition (1 min); incubated at 0–5°C for 24 hrs.	3	3	4	1	4
7	Slow addition (1 min); warm reagents; incubated at 37°C for 30 min; ice box overnight.	0	4	1	1	1
6	Slow addition (1 min); cold reagents; incubated at 0–5°C for 24 hrs.	1	3	1	0	1
Total = 48	Slow addition of At to antiserum.	7	15	12	4	10

[1] 400 microgms. bovine albumin nitrogen contained in 0.2 ml. saline added to 1 ml. of antiserum F_1 (797 microgms. AbN/ml.). This mixture is in considerable antigen excess. See Table IV and Text Fig. 3. Guinea pigs were injected intravenously with 2 ml. of the supernatant fluid obtained by centrifugation.

guinea pigs passively sensitized with antibody and immediately injected with antigen, under which condition a high degree of sensitivity is not ordinarily established (see ref. 13 and Table XVII). The effect of the quality of antibody on the capacity of antibody-excess antigen mixtures to produce shock is now under study.

Summary of Experiments 1–5:

The data for Experiments 1–5 are summarized in Table VI. As is there shown, only 1 of 35 animals receiving supernates from antigen-antibody mixtures containing excess antibody exhibited shock; none of the animals died. Of 162 animals receiving supernates from antigen-antibody mixtures prepared with excess antigen, 133 exhibited varying degrees of shock; 59 of these animals showed severe shock or death; 38 died. It is clear that only the supernates

TABLE VI

Summary of Data on the Effect in Guinea Pigs of Supernatant Fluid from Antigen-Antibody Mixtures

Experiment	Number of Animals Injected With Supernate Containing Ab Excess:						Number of Animals Injected With Supernate Containing At Excess:					
	Total #	No Effect	Slight Effect	Mod. Shock	Severe Shock	Deaths	Total #	No Effect	Slight Shock	Mod. Shock	Severe Shock	Deaths
Table I Ea (Antiserum from alum ppt Ea)	8	8	0	0	0	0	14	2	—	2	4	6
Table II Ea (Antiserum from alum ppt Ea)	8	8	0	0	0	0	26	7	—	6	6	7
Table III Ba (Antiserum from alum ppt Ba)	9	8	0	1	0	0	16	2	7	1	2	4
Table IV Ba (Antiserum from Ba in Freund's adjuvant)	10	10	0	0	0	0	18	1	5	4	0	8
Table V Ba (Antiserum from Ba in Freund's adjuvant)							88	17	31	18	9	13
Total	35	34	0	1	0	0	162	29	43	31	21	38
% with severe reaction or death	$\frac{0}{35} = 0\ \%$						$\frac{59}{162} = 36.4\ \%$					
% with death	$\frac{0}{35} = 0\ \%$						$\frac{38}{162} = 23.5\ \%$					
							$p = <.01$					

from antigen-antibody mixtures prepared with excess antigen produce shock with regularity.

B. Studies on the Factors Responsible for the Production of Anaphylactic Shock by Mixtures of Antibody and Excess Antigen

Experiment 6: The Anaphylactigenic Properties of Supernatant Fluid from Certain Mixtures of Crystallized Bovine Albumin and Gamma-2 Globulin Prepared from Specific Antiserum

The preceding experiments established that the supernatant fluid from antigen-antibody mixtures prepared with excess antigen can induce reactions characteristic of anaphylactic shock in normal unsensitized guinea pigs. The mechanism by which shock was induced was not clear. Several possibilities arose: (a) an "anaphylatoxin" such as that postulated by Friedemann (14), Richet (15), and Friedberger (16, 17) might have been produced *in vitro* by the action of antigen-antibody compounds on the normal serum constituents, (b) the antigen-antibody mixtures might have activated serum proteolytic enzymes as has been suggested by the studies of Bronfenbrenner (18), Jobling and Petersen (19), and Ungar (20, 21), (c) finally, the soluble antigen-antibody complexes produced with mixtures of antibody and excess antigen might in themselves have anaphylactigenic properties. From the onset, the first two

mechanisms appeared unlikely since only the supernates made with antibody and *excess antigen* were capable of producing shock. Studies on the formation of "anaphylatoxin" and activation of proteolytic enzymes by antigen-antibody systems *in vitro* have indicated no clear-cut relationship between these processes and the precipitin curve.

It has been stated that the production of "anaphylatoxin" and the activation of proteolytic enzymes by antigen-antibody reactions *in vitro* require the presence of complement (17, 21). Most of the sera employed in the previous experiments had been aged for several months, so that the complement activity must have been very slight, if present at all. Hemolytic complement activity is not exhibited by gamma-2 globulin fraction of serum. In order to eliminate complement activity and to free the antigen-antibody mixtures of other factors in whole serum which might influence the present observations, antigen-antibody mixtures were prepared with the gamma-2 globulin fraction of a potent antiserum.

Antiserum M was obtained from rabbits receiving 1 subcutaneous injection of 20 mg. of crystallized bovine albumin in Freund's adjuvant every week for a total of 13 weeks. The antibody concentration of this serum was 1.99 mg. antibody N. per ml. The gamma-2 globulin component of 200 ml. of this serum was obtained by ethanol fractionation in the cold as described by Deutsch (22). Paper electrophoresis of this fraction showed gamma-globulin with only a very faint trace of beta-globulin. Antibody content of the fraction was 2.36 mg. Ab N per ml. This represented 56.5 per cent of the total protein contained in the fraction.

Antigen-antibody mixtures were prepared as in previous experiments. The data are summarized in Table VII and presented diagrammatically in Figure 4. Two of 16 animals injected with supernatant fluids prepared either with excess antibody or with equivalent amounts of antibody and antigen showed slight reactions, consisting of slight respiratory difficulty and an occasional cough. However, 10 of 14 animals receiving supernates from antibody-*excess antigen* mixtures showed moderate to severe shock; 2 of these animals died rapidly; 1 died within 3 hours; and 2 others died overnight.

Experiment 7: Further Studies on the Anaphylactigenic Activity of Supernates from Certain Antigen-Antibody Mixtures Prepared with Crystallized Bovine Albumin and Gamma-2 Globulin Fraction of Specific Antiserum

Two hundred ml. of antiserum M was fractionated as in Experiment 6. The resulting gamma-2 globulin preparation contained 2.64 mg. Ab N per ml. This represented 54 per cent of the total protein contained in the fraction. Antigen-antibody mixtures were prepared and the supernates obtained as in Experiment 6.

The results are presented in Table VIII. Nine animals receiving 2 ml. of

TABLE VII

The Effect in Guinea Pigs of the Intravenous Injection of Supernatant Fluid from Antigen-Antibody Mixtures Obtained by the Addition of Varying Amounts of Crystallized Bovine Albumin to 1 ml. of Gamma-2 Globulin (Prep. I) prepared from Specific Antiserum M[1]

No. of Guinea Pigs	Amount of AtN[2] Added to 1 ml. of Antibody Solution, Micrograms	Vol. of Supernatant Fluid Injected; ml.	Supernate Positive for:	No. of Guinea Pigs Showing:				
				No Effect	Slight Shock	Mod. Shock	Severe Shock	Death
Whole Antiserum M								
6	0	2–2.5	Ab	6	0	0	0	0
10	1600	2–2.5	At	0	3	3	2	2
Gamma-2 Globulin M								
5	50	2	Ab	5	0	0	0	0
4	200	2	0	2	2	0	0	0
7	300	2	0	7	0	0	0	0
4	800	2	At	0	0	3	1	0
10	1600	2	At	2	2	1	0	5*

[1] Antiserum M obtained from rabbits receiving 1 injection per week for a total of 13 weeks of 20 mg. bovine albumin in Freund's adjuvant; antibody nitrogen content = 1.99 mg./ml.; antigen equivalence point = 414 microgms AtN. Gamma-2 globulin fraction obtained by alcoholic fractionation. Antibody content = 2.36 mg. AbN/ml. 1 ml. of anti Ba gamma-2 globulin and 1 ml. of antigen dilution gave the following precipitate values:

BaN added, Micrograms	Precipitate N, Micrograms	Supernate Positive for:
101	1502	Ab
350	2663	0
410	2772	0
850	2164	At
1700	86	At

[2] Antigen contained in 0.2 ml. saline.

* 2 died immediately; 1 within 3 hours; 2 overnight, following severe shock.

supernatant fluid containing excess antibody showed no reactions. However, all 11 animals receiving 2 ml. of supernatant fluid containing excess antigen showed symptoms of anaphylactic shock; 3 of these died immediately; 3 others showed progressive weakness and died within 24 hours.

Experiments 8–10: The Effect of the Precipitation of Excess Antigen and Soluble Antigen-Antibody Complexes, by the Addition of Specific Antibody to Supernates, on the Anaphylactigenic Activity of the Supernates

The foregoing experiments strongly indicated that the shock produced by supernates prepared from antibody-excess antigen mixtures was due directly to the antigen-antibody components of the supernate rather than due to the formation *in vitro* of "anaphylatoxin" or the activation of proleolytic enzymes. Firstly, only the supernatant fluid from antigen-antibody mixtures prepared with *excess antigen* were consistently anaphylactigenic; secondly, mixtures

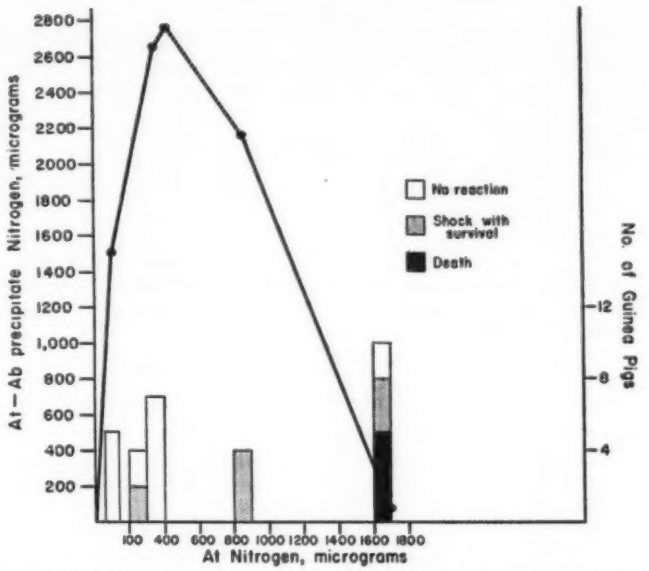

FIG. 4. Relationship between the precipitin curve (Ba-anti Ba γ_2 globulin) and the capacity of supernatant fluid to produce shock.

prepared with purified antibody preparations devoid of complement activity (which seemed to be required for the *in vitro* formation of "anaphylatoxin" and activation of proteolytic enzymes) were still capable of producing shock.

In order to further determine the immunologic basis for the anaphylactigenic activity of the supernates, the effect of precipitation of the excess antigen and antigen-antibody complexes by the addition of specific antiserum to the supernates, on the anaphylactigenic activity of the supernates was investigated. Accordingly, one-half of the pooled supernates from anti-Ea serum and excess Ea, capable of producing shock, were treated with two successive volumes of anti-Ea serum. The treated supernate was now found to contain excess antibody. As a control for possible non-specific absorption of the anaphylactigenic component due to immune precipitation, the other half of the pooled supernate was treated with a heterologous antigen-excess antibody system (Ba-anti Ba system). This treated supernate still contained excess specific antigen (Ea).

It was found that only the original untreated supernate and the supernate treated with the heterologous immune system were capable of producing shock. The supernate in which the excess antigen (Ea) and antigen-antibody complexes had been removed by treatment with specific antibody was no longer capable of producing shock in guinea pigs. The data are summarized in Table IX.

TABLE VIII

The Effect in Guinea Pigs of the Intravenous Injection of Supernatant Fluid from Antigen-Antibody Mixtures Obtained by the Addition of Varying Amounts of Crystallized Bovine Albumin to 1 ml. of Gamma-2 Globulin (Prep. II) Prepared from Specific Antiserum M[1]

No. of Guinea Pigs	Amount of AtN[2] Added to 1 ml. of Antibody Solution, Micrograms	Vol. of Supernate Injected, ml.	Supernate Positive For:	No. of Guinea Pigs Showing:				
				No Effect	Slight Shock	Mod. Shock	Severe Shock	Death
9	220	2	Ab	9	0	0	0	0
11	3400	2	At	0	2	3	0	6*

[1] Gamma-2 globulin obtained by alcoholic fractionation of antiserum from rabbits receiving 13 weekly injections of 20 mg. bovine albumin in Freund's adjuvant. 1 ml. of anti Ba Gamma-2 globulin and 1 ml. of antigen gave following precipitate values:

BaN Added, Micrograms	Precipitate N, Micrograms	Supernate Positive for:
101	1576	Ab
304	2775	—
400	3018	—
487	3124	— (2.64 mg. AbN/ml.)
609	3192	At
800	2979	At
1700	498	At
3400	trace	At

[2] Antigen contained in 0.2 ml. saline.

* 3 died immediately; 3 within 24 hours following moderate to severe shock.

Additional experiments undertaken in the same manner are presented in Tables X and XI. Because of the limitations imposed by volume, the antigen was not completely removed in Table X and the mixture was brought back only to the equivalence point in Table XI. The differences are therefore not as marked as in the preceding experiment where the treated supernate contained excess antibody.

These three experiments are summarized in Table XII. The remarkable difference between the anaphylactigenic properties of supernates treated with specific antibody on one hand and of supernates treated with a heterologous antigen-antibody system on the other hand, indicates that the anaphylactigenic activity of antibody-*excess antigen* supernates results from the antibody-antigen components in solution.

Experiment 11: The Effect in Guinea Pigs of Soluble Antigen-Antibody Complexes Prepared by the Addition of Excess Antigen to Washed Immune Precipitates in the Presence of Alkali

In order to more fully establish whether the factor in mixtures of antibody and antigen responsible for anaphylactic shock was the soluble antigen-antibody complex, a procedure was devised whereby such complexes could be produced in almost pure form. Sternberger and his co-workers (7–9) have shown that precipitated antigen-antibody complexes can be dissociated by

TABLE IX

Effect of Removal of Excess Antigen, by Further Addition of Specific Antibody, from Anaphylactigenic Supernates Derived from Antigen-Antibody Mixtures (Ea Anti-Ea System)

No. of Guinea Pigs	Procedure[1]	Vol. of Fluid Injected, ml.	Inoculum Positive For:	No. of Guinea Pigs Showing:			
				No Effect	Mod. Shock	Severe Shock	Death
(a) 5	1 ml. supernate and 1 ml. saline	2	At	1	2	1	1
(b) 5	1 ml. supernate 0.1 ml. = 150 microgms BaN } heterologous 2 × 0.5 ml. Ba antiserum } At-Ab system	2	At	0	2	1	2
(c) 5	1 ml. supernate 2 × 0.5 ml. Ea antiserum (specific absorption of Ea)	2	Ab	5	0	0	0

[1] Supernate obtained as follows: Excess Ea (150 micrograms N = 0.1 ml.) added to 1 ml. of Ea antiserum (610 micrograms AbN/ml.). Incubated for 48 hrs. at 0–5°C. Centrifugation at 2200 r.p.m. for 30 min. yielded clear supernate. Supernates from each tube were pooled and then redistributed in 1 ml. quantities into clean centrifuge tubes. Thereafter, the following procedures were done:

(a) To a third of the tubes, 1 ml. of saline was added and incubated as in (b)–(c).

(b) To another third of the tubes, 0.1 ml. = 150 micrograms bovine albumin N was added, followed by the addition of 0.5 ml. anti-bovine albumin serum. These tubes were incubated for 1 hr. at 37°C and then another 0.5 ml. of anti-bovine albumin serum was added. After a second incubation for 1 hr. at 37°C, the tubes were centrifuged to get rid of the bovine albumin-antibody precipitate and the supernate used for testing. This entire procedure was carried out to determine the effect of non-specific precipitation on the original supernatant fluid containing excess egg albumin. The final fluid was positive for excess Ea but negative for excess Ba.

(c) The remaining third of the tubes received 2 additions of 0.5 ml. each of Ea antiserum. These tubes were incubated as in (b). The final fluid was negative for excess Ea but positive for Ea antibody.

brief treatment with alkali at low temperature, without appreciable irreversible alteration in the antigen or antibody molecules. Reassociation and precipitation of the antigen and antibody occurs on neutralization. This suggested that soluble antigen-antibody complexes might be formed by dissociation of immune precipitates by alkali in the presence of added (excess) antigen.

To a series of tubes containing twice washed immune precipitates prepared from 1 ml. antiserum J (pooled antiserum containing 2.03 mg. AbN/ml. obtained from rabbits receiving 20 mg. Ba in Freund's adjuvant, weekly for 24 weeks) and 400 micrograms of BaN (equivalence point) were added graded amounts of antigen dissolved in 1 ml. of distilled water.[5] The precipitates were dispersed and dissolved in 0.1 ml. of 1N NaOH. The resulting pH varied from 11.5 to 12.5. Six minutes later, enough IN HCl (usually 0.1 ml.) was added

[5] The antigen was dissolved in water instead of saline in order that the final solution following the addition of 1N NaOH and HCl would be more nearly isotonic.

TABLE X

Effect of Removal of Excess Antigen, by Further Addition of Specific Antibody, From Supernates Derived From Antigen-Antibody Mixtures (Ea Anti Ea Systems). Anaphylactigenic Properties of Treated Supernates

No. of Guinea Pigs	Procedure	Vol. of Fluid Injected, ml.	Inoculum Positive For:	No. of Guinea Pigs Showing:			
				No Effect	Mod. Shock	Severe Shock	Death
(a) 2	Supernate { 1 ml. Ea antiserum and 0.4 ml. = 50 microgms. EaN	2	Ab	2	0	0	0
(b) 4	Supernate { 1 ml. Ea antiserum and 0.4 ml. = 400 microgms. EaN	2	At	1	1	0	2
(c) 6	2 ml. supernate (b) and 4 × 0.5 ml. BGG antiserum and 4 × 0.05 ml. (40 microgms. N) BGG[1]	3.5	At	0	1	0	5
(d) 6	2 ml. supernate (b) and 4 × 0.5 ml. Ea antiserum[2]	3.5	At (*trace*)	4	1	1	0
(e) 2	2 ml. Ea antiserum and 2 ml. BGG antiserum	4.0		2	0	0	0

[1] Supernate treated with BGG antiserum, then BGG antigen, four times with ½ hr. incubation period between additions. Centrifuged and supernate used for testing. Supernate positive for Ea.

[2] Supernate treated with four successive additions of Ea antiserum with ½ hr. incubation period between additions. Centrifuged and supernate used for testing. Supernate showed trace Ea.

TABLE XI

Effect of Removal of Excess Antigen (by Further Addition of Specific Antibody) from Supernates Derived from Antigen-Antibody Mixtures (Ba Anti Ba System). Anaphylactigenic Properties of Treated Supernates

No. of Guinea Pigs	Procedure[1]	Vol. of Fluid Injected, ml.	Inoculum Positive For:	No. of Guinea Pigs Showing:				
				No Effect	Slight Shock	Mod. Shock	Severe Shock	Death
(a) 16	Supernate from excess At-Ab mixtures, treated with heterologous At and Ab	3.5	At	2	5	4	3	2
(b) 15	Supernate from excess At-Ab mixtures, treated with homologous Ab	3.5	Neither At nor Ab	11	3	1	0	0

[1] To 10 tubes containing 10 ml. anti-bovine albumin serum F, (797 micrograms AbN/ml.), 2 ml. = 4.0 mg. BaN were added. Tubes were allowed to sit at room temperature for 1 hour and then centrifuged. Test on the supernate showed considerable antigen excess.

(a) After centrifugation, to 5 tubes 0.5 ml. = .5 mg. of bovine gamma globulin nitrogen was added, followed by the addition of 7.5 ml. of anti bovine gamma globulin serum H (antibody content = 480 microgms AbN/ml.). These tubes were allowed to sit at 37°C for 1 hr. and centrifuged. The supernatant used for injection showed excess bovine albumin but no excess bovine gamma globulin.

(b) To 5 tubes, 7.5 ml. of anti bovine albumin serum J (2.05 mg. AbN/ml.) were added. These tubes were incubated and centrifuged as in (a). The supernatant fluid used for injection did not contain detectable Ba or anti Ba.

TABLE XII

Summary of Experiments on the Effect of the Removal of Excess Antigen and Antibody-Antigen Complexes from Supernates Derived from Antigen-Antibody Mixtures on the Anaphylactigenic Properties of the Supernates

No. of Guinea Pigs	Supernate Derived from Excess Antigen-Antibody Mixtures and Treated With:	Treated Supernate Positive for:	No. of Guinea Pigs Showing:				
			No Effect	Slight Shock	Mod. Shock	Severe Shock	Death
(a) 27	Heterologous At-Ab system in Ab excess	At	2	5	7	4	9
(b) 26	Homologous antibody	Ab or trace At	20	3	2	1	0

% of animals (a) with severe reactions or death = 48.1%
% of animals (b) with severe reactions or death = 3.8% $p < .01$

TABLE XIII

The Effect of Excess Antigen on Immune Precipitates Treated with Alkali

Tube No.	Antigen N	1N NaOH, ml.		1N HCl, ml.	Presence of Precipitate
1	16 mg. in 1 ml.	0.1	6 minutes later	0.1	0
2	8 mg. in 1 ml.	0.1		0.1	opalescent
3	4 mg. in 1 ml.	0.1		0.1	trace
4	2 mg. in 1 ml.	0.1		0.1	+
5	1 ml. water	0.1		0.1	++++

Each tube contained 2 × washed immune precipitate prepared from 1 ml. antiserum J (2.03 mg. AbN) and 400 micrograms of BaN. The antigen or distilled water was added and the precipitate dispersed. The precipitates were dissolved on the addition of NaOH. Six minutes later, enough 1N HCl (usually 0.1 ml.) was added to adjust pH to approximately 7.4, and the tubes were observed ½ hr. later for the presence of immune precipitate.

to adjust the pH to approximately 7.4–7.6. This entire procedure was carried out in the cold. The tubes were observed at room temperature for a half hour to determine the presence of immune precipitation due to the reassociation of antigen and antibody.

The results are recorded in Table XIII. As there shown, the addition of antigen N in excess of 4 mg. completely prevented the reprecipitation of antigen and antibody presumably due to the formation of soluble antigen-antibody compounds; complete reprecipitation only occurred in the tubes which did not receive additional antigen.

To test the activity of the soluble antigen-antibody compounds prepared in this manner, 40 tubes each containing 1 ml. antiserum J and 400 micrograms of BaN in 1 ml. saline were incubated for 45 minutes at 37°C. The resulting precipitates were washed twice with 2 ml. of cold saline and the tubes drained. To each precipitate was added 8 mg. BaN in 1 ml. distilled water. The precipitates were dispersed by shaking and then dissolved in 0.1 ml. 1N sodium hydroxide to a pH of approximately 12.0 Six minutes later, enough 1N hydrochloric acid (approximately 0.1 ml.) was added to adjust to a pH of

aproximately 7.4. The final solutions were very faintly opalescent. The solutions were pooled and injected in 2.4 ml. volumes intravenously into normal guinea pigs. With the volumes used for injection, each guinea pig received approximately 4.06 mg. AbN and 8.8 mg. BaN, presumably in the form of At-Ab complexes and free At. As a control, a similar technique was carried out with 1 ml. of distilled water instead of 1 ml. of antigen solution. Upon neutralization, a heavy immune precipitate resulted. This mixture was centrifuged and the supernate injected in 2.4 ml. volumes. Following neutralization, most of the antigen and antibody had recombined for the clear supernate contained only 25 micrograms of protein N per ml. (presumably due to the slight solubility at room temperature of the immune precipitate).

The results are recorded in Table XIV. None of 10 animals injected with the supernates prepared from immune precipitates treated with alkali in the absence of excess antigen and subsequently neutralized showed any ill-effects. However, 13 of 15 animals treated with the supernates prepared from immune precipitates treated with alkali and held in solution following neutralization because of the presence of additional antigen showed signs characteristic of anaphylactic shock.

TABLE XIV

The Effect in Guinea Pigs of Soluble Antigen-Antibody Complexes Prepared by the Addition of Excess Antigen to Washed Immune Precipitates in the Presence of Alkali

No. of Guinea Pigs	Treatment[1]	Vol. Injected, ml.	No. of Guinea Pigs Showing:				
			No Reaction	Slight Reaction	Moderate Shock	Severe Shock	Death
15	(a) With soluble complexes prepared from immune precipitates and excess antigen	2.4	2	5	6	1	1
10	(b) With supernatant fluid from immune precipitates without excess antigen	2.4	10	0	0	0	0

[1] Procedure for preparation of (a) soluble complexes and (b) fluid used as control:

(a) To 40 tubes each containing 1 ml. antiserum J (Eq point = 387 micrograms BaN) was added 400 micrograms BaN. After incubation for 45 min. at 37°C, tubes were centrifuged and the resulting precipitates twice washed with cold saline and recentrifuged. The resulting precipitate contained 2.03 mg. AbN and approximately 400 micrograms of BaN. To each drained precipitate was added 8 mg. BaN in 1 ml. distilled water. The precipitates were dispersed and then dissolved in 0.1 ml. 1 N sodium hydroxide at which time the pH was approx. 12.0. In approximately 6 minutes, enough 1 N HCl was added to adjust to a pH of 7.4. The final solutions were slightly opalescent. The solutions were pooled and injected in 2.4 ml. volumes intravenously into guinea pigs. All of the above techniques were carried out at 0°C. With the volumes used for injection, each guinea pig received approximately 4.06 mg. AbN and 8.8 mg. BaN, presumably in form of At-Ab complexes and free At.

(b) As a control for (a) a similar technique was carried out without the presence of additional antigen (1 ml. of distilled water was added to the washed precipitates instead of 1 ml. of antigen solution). On the addition of enough 1 N HCl to reduce the pH of 12.5 to 7.4, a heavy immune precipitate resulted. The mixture was centrifuged and the supernate injected as above. This supernate contained only 25 micrograms of protein N per ml.

Experiment 11A: The Effect in Guinea Pigs of Soluble Antigen-Antibody Complexes Prepared by Mechanical Agitation of Washed Immune Precipitates with Excess Antigen

To avoid the use of alkali, the present experiment was undertaken with soluble antigen-antibody complexes prepared simply by the mechanical agitation of washed immune precipitates with excess antigen.

Preliminary observations indicated that the addition of 16 mg. At N to washed immune precipitates would result in dissolution of the precipitates following shaking for 24 hours. Accordingly, soluble antigen-antibody complexes were prepared as outlined in Table XV. As shown, when 2 ml. of the solution consisting of 3.75 mg Ab N and 16.8 mg At N were injected into each of 12 guinea pigs, all showed signs of anaphylactic shock.

C. Preliminary Studies on the Mechanisms by which Soluble Antigen-Antibody Complexes Induce Shock

Experiment 12: A Comparison of the Amount of Antibody Required for Passive Anaphylaxis in Guinea Pigs when Challenged Immediately with Excess Antigen, with the Amount of Antibody in Antibody-Excess Antigen Supernates Capable of Producing Shock

The previous experiments indicated that the anaphylactigenic activity of supernates from antibody-excess antigen mixtures results from the presence of soluble antigen-antibody complexes and free antigen. The presence of soluble antigen-antibody complexes in mixtures containing antibody and excess antigen has been demonstrated by electrophoretic and ultracentrifugal studies (23–27).

TABLE XV

The Effect in Guinea Pigs of Soluble Antigen-Antibody Complexes Prepared by Agitation of Washed Immune Precipitates in the Presence of Excess Antigen[1]

No. of Guinea Pigs	Volume Injected, ml.	No. of Guinea Pigs Showing:				
		No Reaction	Slight Shock	Moderate Shock	Severe Shock	Death
12	2.0	0	2	6	1	3*

[1] To 29 tubes containing 1 ml. antiserum M (obtained from rabbits receiving 1 injection per week for a total of 13 weeks of 20 mg. bovine albumin in Freund's adjuvant; antibody nitrogen content = 1.99 mg./ml.; antigen equivalence point = 414 micrograms At N) were added 400 micrograms of BaN in 1 ml. saline at room temperature. Five minutes later, the tubes were centrifuged at 2,000 r.p.m. for 10 minutes only, to avoid firm packing of the precipitate. The precipitates were washed with 2 ml. of cold saline, centrifuged for a short time and drained. To the dispersed precipitates were added 1 ml. = 16 mg. BaN in saline. The tubes were shaken for 24 hours in the cold. On centrifugation, only a trace of precipitate was obtained. The clear supernate was injected into guinea pigs in 2 ml. volumes. Kjeldahl determinations on the original twice washed precipitate showed that it contained 1.88 mg. AbN. Therefore the animals received approximately 3.76 mg. AbN and 16.8 mg. BaN in the form of soluble antigen-antibody complexes.

* Two animals died acutely; one died within 6 hours after showing moderate shock.

The signs during life and the pathologic findings in animals shocked by supernates from antibody-*excess antigen* mixtures were identical in all respects to those of "classical" anaphylactic shock produced by the injection of antigen into a sensitized animal. In order to further clarify the relationship between shock produced by immune supernates and anaphylactic shock produced by the classical method of injecting antigen into a previously sensitized animal, an investigation was made into the quantitative immunological aspects of these two phenomena.

(a). The estimation of the amounts of antibody and antigen in the supernates of antibody-excess antigen mixtures producing shock.

In order to determine the amounts of antigen and antibody in the supernates of antibody-excess antigen mixtures, it is necessary to know the amount of antigen and antibody which have been carried down in the precipitate. This ordinarily requires the use of labelled antigens (5). Observations by other investigators have suggested that with the antigen-antibody systems used in the present study the molecular ratio of antibody to antigen in the precipitate formed in antigen excess is usually of the order of Ab_2 At (5). This assumption was used in determining the antibody and antigen content of the precipitates produced in antigen excess in the present study. For the Ea-anti Ea system this molecular ratio is equivalent to a weight ratio of AbN to AtN of approximately 7:1; for the Ba-anti Ba system, this molecular ratio is equivalent to a ratio of AbN to AtN of approximately 4:1; assuming that the molecular weights of Ea, Ba and rabbit antibody are 42,000; 69,000; and 150,000 respectively. Knowing the amount of antibody in the precipitate, the amount of antibody in the supernate can be calculated by subtracting the amount of antibody in the precipitate from the total amount of antibody added. The amount of antigen in the supernate can be calculated in a similar manner. The results of these calculations carried out on the antibody-excess antigen mixtures of the previous experiments are presented in Table XVI. In the last 2 columns are presented the amounts of antibody and antigen contained in the volumes of supernate effective in producing shock. As there shown, the supernates capable of producing shock contained amounts of antibody ranging from .119 mg. AbN to 3.72 mg. AbN, presumably in combination with antigen.

(b). The determination of the amounts of antibody required for immediate passive sensitization to a challenging injection of excess antigen.

In many early studies on passive anaphylaxis, it was found that a certain latent period of several hours must follow the injection of antibody before the animals can be shocked by antigen (28, 29). Recently it has been shown that the latent period required for passive sensitization is a function of the amount of antibody injected (13). With large amounts of antibody, immediate sensitization can be produced (13). Since the shock produced by antibody and antigen

TABLE XVI

The Antibody and Antigen Content (in the form of At-Ab complexes and free At) of Supernatant Fluid Producing Anaphylaxis

Experiment From:	Total AbN/ml. of Antibody Preparation,[1] Micrograms	Amount of AtN Added to 1 ml. of Antibody Preparation Micrograms	Total ppt. N Micrograms	Amount of[2] AbN AtN in Precipitate, Micrograms		Amount of[3] AbN AtN in Supernate, Micrograms		Amount of AbN AtN in Volume of Supernate Injected, Micrograms	
				AbN	AtN	AbN	AtN	AbN	AtN
Table I (Ea)	794	180	649	569	81	231	99	231	99
		240	457	400	57	394	183	394	183
Table II (Ea)	533	150	475	416	59	119	91	119	91
		200	385	337	48	198	152	198	152
		400	163	143	20	392	380	392	380
		800	87	76	11	459	789	459	789
Table IV (Ba)*	797	200	862	690	172	107	28	178	47
		410	53	42	11	755	399	1253	662
Table VII (Anti-Ba Gamma-2 Globulin)	2362	800	2164	1729	435	633	365	1055	610
		1600	86	69	17	2293	1583	3722	2638

[1] Antibody preparation refers to antiserum or gamma-2 globulin fraction.

[2] Molecular ratio of Ab:At of the precipitate formed in antigen excess was assumed to be Ab_2 At; this is approximately equivalent to a ratio of AbN to AtN of 7:1 for the Ea system and 4:1 for the Ba System.

[3] Obtained by subtraction of amount of Ab and At in precipitate from amount of Ab and At added.

* All supernates were injected in 2 ml. volumes. Antigen antibody mixtures prepared in experiments above this point by the addition of 1 ml. of antigen solution to 1 ml. of antiserum; in experiments below this point 0.2 ml. of antigen solution to 1 ml. of antiserum was used. Thus, the difference in columns 6 and 7.

in complex formation in immune supernates is immediate, an experiment was undertaken to determine the amounts of antibody which would give rise to immediate shock in guinea pigs when challenged with excess antigen as quickly as possible after the injection of the free antibody.

For this purpose, an experimental procedure was employed similar to that described by Benacerraf and Kabat (13). Both hind legs of normal guinea pigs were exposed. Antibody (anti Ba) in whole or diluted antiserum was injected in a 1 ml. volume into one leg vein and within 20 seconds, antigen (Ba) was injected into the other leg vein.

As shown in Table XVII, amounts of antibody ranging from 0.1 mg. to 2.0 mg. were capable of producing immediate sensitization of guinea pigs to a challenging injection of antigen. Deaths were observed with as little as 0.25 mg. AbN. However, it is of interest that even with 2.0 mg. AbN, death did not uniformly occur from anaphylactic shock. The great variation in the response of animals to the injection of free antibody immediately followed by the injec-

TABLE XVII

The Effect of Dosage of Antibody on Passive Anaphylactic Shock Produced by the Intravenous Administration of Antiserum Followed Immediately by the Intravenous Injection of Excess Antigen[1]

No. of Guinea Pigs	Antibody N Injected, mg.[2]	At N Injected, mg.	No. of Guinea Pigs Showing:				
			No Effect	Slight Shock	Moderate Shock	Severe Shock	Death
8	2.0	.812	0	2	1	1	4*
5	2.0	.406	0	1	0	1	3
10	0.5	.203	2	4	1	0	3*
13	0.25	.100	2	5	3	1	2
12	0.10	.100	2	5	2	3	0

[1] Antiserum M was obtained from rabbits receiving 1 injection per week for a total of 13 weeks of 20 mg. bovine albumin in Fruend's adjuvant. Antibody nitrogen content = 1.99 mg./ml.; antigen equivalence point = 414 micrograms AtN.

[2] Antiserum (Anti-Ba) injected in one leg vein, antigen (Ba) injected in other leg vein. The antigen and antibody solutions were contained in 1 ml. volumes. The interval between completion of injections was 15 to 25 seconds.

* 2 with delayed deaths.

tion of antigen is similar to the great differences observed in the reaction of animals challenged with antigen-antibody supernates. The results of experiments (a) and (b) suggest that the amounts of antibody contained in antigen-antibody supernates capable of producing shock is of the same order of magnitude as the amounts of free antibody required to produce instantaneous anaphylactic shock in guinea pigs when antibody is immediately followed by antigen.

Experiment 13: The Effect of Prior Injection of Large Amounts of Antigen into Guinea Pigs on Shock Produced by Immune Supernates

A consideration of the immunologic mechanisms by which supernates containing antigen-antibody complexes and free antigen could produce shock in animals led to several possibilities. Firstly, following the intravenous injection of the supernates, it is possible that antigen-antibody complex might dissociate when the free antigen with which it is in equilibrium (23, 24) is diluted by the plasma and tissue fluids. Shock might then be produced by the recombination of antibody with antigen in the tissues. Secondly, shock might result from the precipitation of antigen-antibody complexes in the circulation as the equilibrium between free antigen and the complex is disturbed by dilution. Thirdly, antigen-antibody complexes in themselves might be capable of producing shock by acting on susceptible body cells.

In order to obtain information on the first two possibilities, guinea pigs were injected with a large amount of antigen (300 mg. Ba) 15 minutes prior to the shocking injection of the supernate. It was believed that such large amounts of antigen in the body would insure against any possible dissociation or precipitation of the antigen-antibody complex when the shocking supernate was

TABLE XVIII

The Effect of Prior Treatment of Guinea Pigs with Antigen on the Anaphylactigenic Capacity of Supernatant Fluid Obtained from Antibody-Excess Antigen Mixtures[1]

No. of Guinea Pigs	Prior Treatment	No. of Guinea Pigs Showing:				
		No Effect	Slight Shock	Moderate Shock	Severe Shock	Death
9	None	3	2	1	0	3*
9	3 ml. = 300 mg. Ba i.v. 15 min. before injection of supernate	2	1	3	0	3

[1] Gamma-2 globulin obtained by alcoholic fractionation of antiserum from rabbits receiving 13 weekly injections of 20 mg. crystallized bovine albumin in Freund's adjuvant. Precipitate values with increasing quantities of BSA are presented in appendage to Table VIII. To a number of tubes containing 1 ml. gamma-2 globulin was added 3400 micrograms of BaN (antigen in extreme excess) contained in 0.2 ml. saline. The tubes were allowed to stand in the cold for 48 hours and then centrifuged. Each animal was injected with 2 ml. of the supernatant fluid. Half of the animals received 3 ml. = 300 mg. Ba intravenously 15 minutes prior to injection of the supernates. In order to exclude the possibility that the shock obtained in the animals previously injected with antigen was not due to free antibody in the supernatant, directed against an impurity, the animals which received no antigen and survived following shock with supernates were injected with 3 ml. of Ba = 300 mg. on the following day. None of these animals showed untoward effects. It can therefore be concluded that the shock in the animals previously treated with antigen was due to the activity of the supernates.

* 1 showed delayed reaction with death within 24 hours.

diluted by the body fluids. The results of this experiment are recorded in Table XVIII. As shown there, prior treatment with large amounts of antigen (many times in excess of that used in the test-tube) did not alter the reaction of the animals to the supernatant fluids. As a control, in order to exclude the possibility that the shock obtained in the animals previously injected with antigen was due to free antibody in the supernate directed against an impurity or more remotely against Ba itself, the surviving animals which had received only the supernates were injected with large amounts of antigen 24 hours later. None of these animals showed symptoms of shock, even though as small an amount as 30 micrograms of antibody N will induce almost uniformly fatal anaphylaxis with an incubation period of 24 hours between the injection of antibody and antigen (13, 30). The failure of the prior treatment of animals with large amounts of antigen to inhibit shock produced by immune supernates suggests that the shock following the injection of immune supernates is not due to dissociation or precipitation of the immune complex *in vivo*.

D. Studies on the Effects of Insoluble Antigen-Antibody Complexes on Normal Guinea Pigs

Experiment 14: The previous experiments were undertaken with clear supernatant fluids of antigen-antibody mixtures. It was deemed important to ascertain the effect of the intravenous injection of antigen-antibody precipitates.

For this purpose, to tubes containing 1 ml. of antiserum M containing 1.99 mg. of Ab N per ml. were added 400 micrograms of BaN in 0.2 ml. saline. The supernate was negative for both antigen and antibody. The supernates were discarded and the precipitate obtained from each tube by centrifugation was finally suspended in 1 ml. of saline. Ten animals were injected intravenously with 2 ml. of this suspension, equivalent to 4.0 mg. AbN and 0.8 mg. AtN in aggregated form. Two showed an occasional cough and slight respiratory distress. All of the others exhibited ruffled fur, sighing respiratory movements and slight weakness. Except for the two animals exhibiting cough, none of the animals showed the chain of signs characteristic of anaphylactic shock, even though the injected precipitates contained large amounts of combined antibody and antigen.

It is concluded that insoluble antigen-antibody complexes do not give rise to typical anaphylactic shock. This conclusion is supported by the anatomic findings on 5 of the above animals sacrificed within 10 to 15 minutes after injection. Unlike the animals injected with soluble antigen-antibody preparations, where the lungs show marked emphysema, the lungs of these animals were not overinflated. Further, microscopic examination showed numerous amorphous hyaline and granular thrombi plugging the alveolar capillaries. Although the nature of the thrombi is uncertain, it seems likely that they represent particles of the immune precipitate caught in the small capillaries of the lungs (31).

DISCUSSION

The mechanism by which antigen-antibody reactions induce anaphylactic shock has received the attention of numerous investigators ever since this phenomenon was first widely appreciated following the observations of Richet, Theobald Smith and Otto (15, 28, 29). Two opposing theories have developed to explain the phenomenon of anaphylaxis.

One of these maintains that the reaction between antigen and antibody, occurring in the blood and the extravascular fluid, gives rise to a toxic substance which is capable of producing shock by its action on susceptible body cells. This theory was developed by Friedberger (16, 17) following the observation by Friedemann (14) that normal rabbits developed all of the symptoms of anaphylactic shock following the injection of a mixture of antigen (beef blood) and immune serum which had been incubated for a short time. This hypothetical toxin was called "anaphylatoxin" by Friedberger (16) and "apotoxin" by Richet (15).

It was soon found that sera could be rendered toxic by a variety of substances other than antigen-antibody mixtures; e.g., kaolin (32), barium sulfate (33) and agar (34). These observations led to the belief that the toxicity of serum treated with insoluble antigen-antibody precipitates or chemicals was due to

the absorption by these substances of the serum anti-tryptic ferments so that the tryptic ferments were free to act on the serum proteins (18, 19). This mechanism has been employed to explain both anaphylactic shock and anaphylactoid shock which can be produced by a variety of chemicals (20, 21, 35, 36).

According to another theory (37), anaphylactic shock is not due to the formation of a toxic substance following antigen-antibody combination in the blood but results from a cellular disturbance initiated by the reaction of antigen with antibody anchored to cells. In most instances, when animals are passively sensitized by the injection of immune sera, a certain interval of time must elapse before animals become susceptible to a shocking injection of antigen. This phenomenon has been interpreted as indicating that the antibodies passively introduced were effective in producing hypersensitivity only after they had become "fixed" to the body cells (28, 38). The work of Schultz (39) and Dale (40) indicating that isolated smooth muscle from a sensitized animal would contract on the addition of the specific antigen gave further support to the contention that the antibody involved in anaphylaxis was that which was fixed to cells. The observation of Dale and Laidlaw (41) that histamine when injected caused symptoms similar to anaphylactic shock led to the current popular concept that anaphylactic shock is produced by the liberation of histamine when antigen unites with antibody fixed to body cells.

Little attention has been given to the role of antigen-antibody complexes in anaphylactic shock. Gladstone (42) states, "The anaphylatoxin is not the antibody-antigen compound itself, for a neutral mixture of antigen and antibody will not cause contraction of the guinea pig uterus". It is difficult to relate the present findings with the older observations on "anaphylaxis *in vitro*" because less was known then of the nature of antigen-antibody reactions; homogeneous antigens were not used and the quantitative techniques for the determination of antigen and antibody had not been developed.

The results of the present study indicate that mixtures of antibody with excess antigen are capable of producing anaphylactic shock in normal unsensitized guinea pigs. The data indicate that the shock is due to the action of soluble antigen-antibody complexes. First, but for minor exceptions, only the supernatant fluid from antibody-antigen mixtures prepared in antigen excess are capable of producing shock. Secondly, when the excess antigen in free form and in complex with antibody in the supernate is removed by treatment with additional antibody, the anaphylactigenic capacity of the supernate is abolished. Thirdly, supernates derived from mixtures of purified antibody (gamma-2 globulin) and excess antigen are capable of producing shock. Fourthly, soluble antigen-antibody complexes produced by the dissociation of washed immune precipitates by alkali or by mechanical agitation in the presence of excess antigen exhibit anaphylactigenic properties. Fifthly, the amounts of antibody in complex formation with antigen capable of producing

shock are similar in magnitude to the amount of antibody producing shock in animals when injected in free form and immediately followed by antigen (passive anaphylaxis without incubation period).

The character of the shock produced by soluble antigen-antivody complexes is identical with that produced by ordinary passive or active anaphylaxis. The lungs of animals dying rapidly showed marked emphysema and an occasional hemorrhage. No pulmonary edema or pulmonary thrombi were observed. Instead of immediate shock, there occurred in some animals a delayed reaction which occasionally led to death within several to 24 hours. Anderson (11) and Weil (12) showed that a delayed reaction may occur in ordinary passive anaphylaxis under conditions where sufficiently large amounts of antigen and antibody are not brought into sudden contact. The present study shows that delayed deaths may also occur in passive anaphylaxis when the antibody and antigen are injected without an intervening "incubation period".

Benacerraf and Kabat (13) have shown that the "incubation period" of passive anaphylaxis is a function of the quantity of antibody injected into the animal. The fact that uniform sensitization can be produced by small amounts of antibody only after an "incubation period" has been interpreted as indicating that antibody must be "fixed" to cells for the production of anaphylactic shock by a challenging injection of antigen. However, there is an alternative interpretation; that is, that since the biochemical reaction producing anaphylaxis takes place in cells, the rapidity and severity of the anaphylactic response may depend not on antibody "fixation" but on the rate at which sufficient amounts of antigen and antibody, either free or in combination, enter the vicinity of the susceptible cells. Such a mechanism would favor the more prompt development of allergic reactions when antibody already entered the tissues before a challenging injection of antigen, and at the same time, would be consistent with the view, supported by the present observations, that antigen and antibody may interact at a distant site and with time, dependent upon the rate of diffusion of the complex, enter the tissues and produce allergic reactions. It is possible that the rigid belief that antibody must be "fixed" to cells in order to produce allergic reactions has developed because of the fact that in anaphylactic shock in contrast to protracted anaphylaxis, the element of time is so essential.

The exact mechanism by which soluble antigen-antibody complexes produce anaphylactic shock is not clear. Because of the strong affinity of antigen for antibody, antibody does not ordinarily exist in free form in the presence of excess antigen sufficient to produce soluble antigen-antibody compounds. Since soluble antigen-antibody complexes are held in solution by excess antigen, the possibility arises that anaphylactic shock following the injection of antibody-excess antigen solution results from a dissociation of the antigen-antibody complexes in the circulation (as the concentration of the excess antigen is

lowered by dilution in the plasma) followed by recombination of the dissociated complexes in the tissues. However, a previous injection of antigen in quantity which would seem adequate to stabilize the antigen-antibody complex *in vivo* did not alter the anaphylactigenic capacity of the antigen-antibody solution. Therefore, this mechanism appears unlikely.

Although the capacity of antigen-antibody solutions to produce allergic reactions in the experimental animal has not been appreciated, it is well established that certain other allergic and immunologic phenomena may be initiated by mixtures of antigen and antibody. In 1927, Gay and Chant (43) showed that the injection of mixtures of human reaginic antibody and specific antigen into human skin produced an immediate skin reaction. Their observations have been substantiated by the work of numerous other investigators (44–47). Boyden and Andersen (48) have shown that certain mixtures of tuberculo-protein and rabbit antiserum to the tubercle bacillus can cause the agglutination of normal sheep cells and, in the presence of complement, produce hemolysis.

During the development of the allergic lesions of protracted anaphylaxis ("serum sickness"), the antigen is in excess of antibody so that soluble antigen-antibody complexes have an opportunity to develop. It seems quite likely that the tissue lesions of protracted anaphylaxis result from the action of these complexes. Studies are now in progress to determine whether the prolonged administration of soluble antigen-antibody complexes into normal rabbits will lead to the development of cardiovascular and renal lesions.[6]

Whether all types of antibody can give rise to soluble antigen-antibody complexes capable of initiating allergic reactions is not clear. Work is now in progress in this laboratory to determine the relative ability of soluble complexes prepared with precipitating antibody on the one hand and non-precipitating antibody on the other hand to produce anaphylactic shock[6]. Since non-precipitating antibody forms soluble complexes with antigen even in antibody excess, it is possible that under certain conditions, this antibody may play the major role in the initiation of allergic reactions.

The present study indicates that under ordinary circumstances, immune precipitates are inactive in producing anaphylaxis. However, upon injection, they are entrapped in the pulmonary capillaries. Animals receiving precipitates may exhibit restlessness and fatiguability, possibly because of obstruction to the pulmonary circulation. It has often been stated that a high circulating

[6]A preliminary experiment has been carried out with Dr. Abou D. Pollack and Dr. George E. McKinnon. Two rabbits were injected intravenously with supernates prepared from antibody-excess antigen mixtures, which were capable of shocking guinea pigs. The antibody was contained in rabbit gamma-2 globulin prepared by ethanol fractionation. The rabbits were infused at a rate of approximately 11 ml. of supernate per hr. for 12 and 24 hours respectively. They were sacrificed 36 hours after the start of the infusion. Both showed slight glomerulonephritis and one showed widespread early necrotizing pulmonary arteritis.

antibody titer acts to inhibit anaphylactic shock (42). It is possible that this inhibition might result from the formation of insoluble complexes between the antigen and excess antibody.

SUMMARY

The supernatant fluid from antigen-antibody mixtures prepared in *antigen excess* produces anaphylactic shock when injected into normal unsensitized guinea pigs. But for minor exceptions the supernatant fluid from antigen-antibody mixtures prepared in *antibody excess* do not give rise to symptoms of anaphylactic shock.

Evidence is presented which demonstrates that the shocking property of antigen-antibody mixtures is due to the presence of soluble antigen-antibody complexes.

Anaphylactic shock does not require the actual interaction of antigen and antibody in the tissues but may be produced by preformed antigen-antibody complexes acting on susceptible cells.

Since the allergic lesions of protracted anaphylaxis develop while there is excess antigen in the circulation, it is likely that soluble antigen-antibody complexes are responsible for the initiation of these lesions.

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PLATE I

FIG. 1. A comparison of the gross appearance of lungs from a normal guinea pig and from one dying within several minutes following a shocking injection of supernate from antibody-excess antigen mixture. The heart and lungs on the left were removed from a normal guinea pig killed by a blow on the head. The hemorrhagic areas are the result of aspiration of blood. On the right are the heart and lung from the animal dying in shock. Note the increase in size due to extensive emphysema. The heart is hidden from view by the overinflated lungs. Several small pulmonary hemorrhages can be seen. This appearance is characteristic of all the lungs removed from animals dying rapidly from antibody-excess antigen solutions. 5× 1.0.

FIG. 2. Microscopic section of lungs of animal dying rapidly from soluble antigen-antibody complexes. (from lungs on the right in Fig. 1). The alveolar spaces are distended; the alveolar walls are delicate. Hematoxylin and eosin. × 160.

FIG. 3. Microscopic section of lungs from a guinea pig killed 10 minutes after the injection of antibody-antigen precipitates. The lungs were not grossly emphysematous. Note the amorphous masses of precipitate in the alveolar capillaries. Hematoxylin and eosin. × 160.

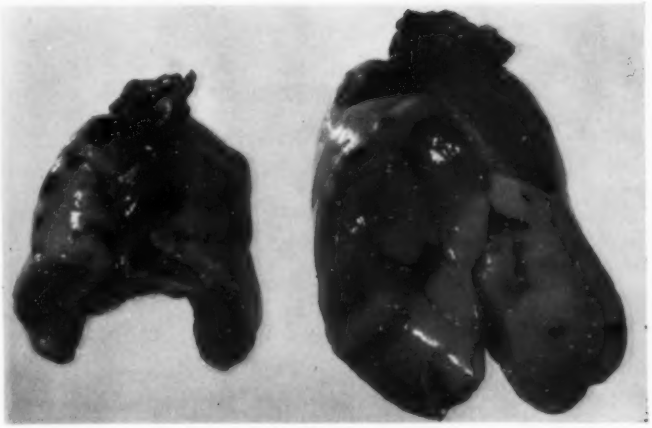

FIG. 1

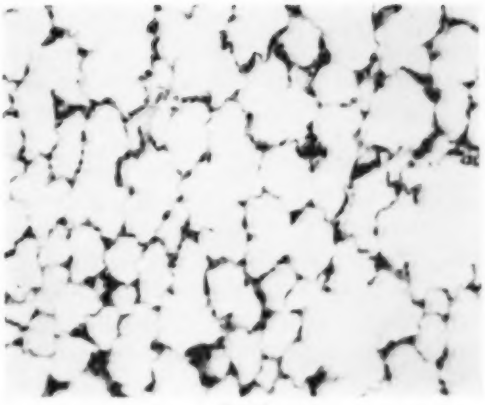

FIG. 2

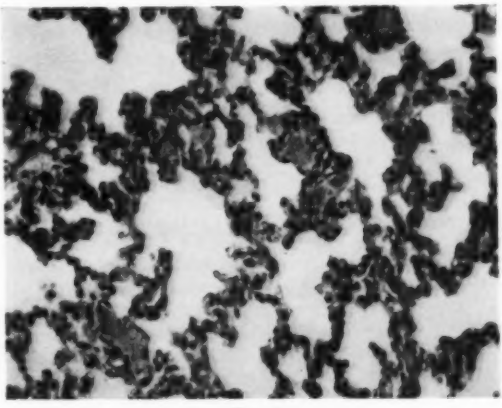

FIG. 3

FETAL BLOOD STUDIES [1]

VI. The Oxygen Capacity of Human Fetal Blood in Term Pregnancy and in Postmaturity

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A. INTRODUCTION

It is generally accepted that the developing fetus is exposed to a reduced partial pressure of oxygen during its stay in the uterus. Accommodation is accomplished by several mechanisms. Some of these are well known; others are less well understood. For many years it has been recognized that one of the methods by which the fetus responds is by increasing the oxygen capacity of its blood. All who have done research on this subject agree on the basal fact that the hemoglobin content or, in other words, the oxygen capacity rises as fetal age advances. In 1934 Elliott *et al.* (1) showed that this was true in the goat, while in 1935 and 1936 Wintrobe and Shumacker (2, 3) made similar observations on the pig, rat, rabbit, cat, dog, and man.

It is also well established that the oxygen capacity of human fetal blood at the end of pregnancy is much higher than that of the mother and higher indeed than is normally observed in extra-uterine existence. In an attempt to elucidate this phenomenon many have compared the fetus *in utero* to an adult living in the rarified air of high mountains. The rise in hemoglobin value experienced by dwellers at high altitudes is well known and the greater degree of relative hypoxia, the higher the hemoglobin value. This analogy has been put forward by many workers as an explanation of the high oxygen capacity of fetal blood at the end of pregnancy (4).

Recently Walker and Turnbull (5) have reported that a fetus which is subjected to a longer stay in the uterus than usual responds by further increasing its oxygen capacity. This observation has led to the belief that postmaturity is associated more or less regularly with hypoxia. The purpose of this paper is to report data on the oxygen capacity of fetal blood in prolonged pregnancy in comparison to its oxygen capacity in pregnancies of normal duration.

B. MATERIAL AND METHOD

Observations are reported on 142 newborn, all delivered on the obstetrical service of the Johns Hopkins Hospital. Shortly after delivery a sample of blood was collected from the

[1] This work is a continuation of previous studies published in this periodical by one of us (N.J.E.) many years ago. The investigation was supported in part by a research grant, H-2885, United States Public Health Service.

TABLE I

Duration of Gestation and Oxygen Capacity

Duration of Gestation (weeks)	No. of Cases		Range of Variation of O_2 Capacity (vols. per cent)		Average O_2 Capacity (vols. per cent)	
36	9		16.6–24.1		20.1	
37	9		18.5–25.1		22.0	
38	16	88	17.6–25.5	16.6–25.5	20.7	20.8
39	24		17.0–24.2		19.9	
40	30		17.8–23.2		21.3	
41	14	54	16.8–25.6	16.2–25.6	21.3	20.9
42 and over	40		16.2–24.5		20.7	

umbilical vein in a heparinized and oiled syringe. The sample was placed in a 250 ml. tonometer filled with air, rotated manually for three minutes to expose the blood in a thin film and oxygen determinations were then performed by the method of VanSlyke and Neill (6). Duplicate determinations in which rotation was carried out for periods varying between five and ten minutes did not show any increased uptake of oxygen. After the determinations were made the patients' charts were obtained from the hospital record room and pertinent data recorded. Only normal pregnancy is considered in this report.

C. RESULTS

As indicated in Table I, 88 of the 142 infants were born of parturients who were 36 to 40 weeks pregnant. In 54 patients (38 per cent), gestation had extended beyond term and ranged from 41 to 45 weeks. Cases of pregnancy in the latter category have been designated as one group, for it is these which previous workers have referred to as "postmature".

In Table I the range of variation and average oxygen capacity is correlated with the gestational age of the infant. The ranges of variation in each weekly period are fairly wide, and in each category they are similar. If one were to consider those pregnancies terminating at 36 to 40 weeks as a group, the values of the oxygen capacity vary from 16.6 volumes per cent to 25.5 volumes per cent. Similarly, if postdate pregnancy be grouped into one category, the range of variation is practically the same, 16.2 to 25.6 volumes per cent. The *average* values at each weekly period reveal the same result. In addition, if the two large groups be compared, the average oxygen capacity is almost identical.

D. DISCUSSION

In the present series of cases prolongation of gestation did not influence the oxygen capacity of fetal blood. This does not necessarily exclude the presence of hypoxia in these post-term fetuses; but if present the hypoxia does not reflect itself in any compensatory increase in hemoglobin.

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FETAL BLOOD STUDIES[1]

VII. The Oxygen Pressure Gradient Between the Maternal and Fetal Bloods of the Human in Normal and Abnormal Pregnancy

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A. INTRODUCTION

The observations that form the substance of this report deal with circumstances associated with the transfer of oxygen across the hemochorial placenta of the human. They were made in an effort to expand our knowledge of the physiology of the exchange of respiratory gases between mother and fetus. According to Grosser's (1) classification, there are three tissue layers in the human placenta—the endothelial lining of the fetal capillary, the fetal connective tissue, and the fetal epithelium; these separate the fetal and maternal plasmas through which the respiratory gases must diffuse. All materials that move from one circulation to the other must cross this barrier.

In the case of the placenta some assumptions and simplifications are essential to an analysis of the transfer of oxygen in physical terms. Let us assume first that the placental barrier can be represented for our purpose by a liquid impregnated membrane—one in which the liquid phase is continuous from surface to surface. Then the volume of gas that will diffuse through the liquid layer per unit of time (Q) will be proportional to: 1. The difference in the pressure of gas on the two sides of the layer ($P_{Mother} - P_{Fetus}$); 2. The area of the smaller surface at right angles to which the diffusion takes place (a); 3. A constant for the system (k). But it will be inversely proportional to the thickness of the layer (d). In equation form this may be expressed

$$Q = \frac{(P_M - P_F)\,(a)\,(k)}{(d)} \qquad (1)$$

In the case of the placenta there is at present no satisfactory method of estimating (d) nor is there any reliable method for estimating (a) or (k). The same difficulty, a lack of knowledge of the dimensions of the barrier separating the air and blood, faced those concerned with the problems of gas transfer in the lungs. Regardless of the inability to measure the above mentioned factors, it should be made quite clear that it is possible to estimate the oxygen gradient; and, other circumstances being equal, the oxygen supply—the rate at which oxygen reaches the fetal blood—will be proportional to that gradient. Hence, there is an interest *per se* in the order of magnitude of the difference in the partial

[1] This investigation was supported in part by research grant, H-2885, U.S.P.H.S.

pressure of oxygen between the maternal and fetal circulations in any individual and the range of variation in different individuals.

A review of the literature reveals there are no previous data on this aspect of placental transfer in the human. Other studies have been concerned with oxygen relationship between maternal peripheral or uterine vessels and the umbilical circulation at the time of delivery. By virtue of the fact that the exchange of oxygen and carbon dioxide occurs across the internal surfaces of the fetal and maternal vascular channels of the placenta proper, the data are of limited value in the study of oxygen transfer. Hence, it was thought desirable to investigate this problem by analyzing blood from the intervillous space which represents the blood to which the fetal blood *in utero* is exposed and the umbilical circulation (Chart 1).

B. MATERIAL AND METHODS

The fourteen patients upon whom observations were made were delivered on the Obstetrical Service at the Johns Hopkins Hospital. The abnormal cases were selected only insofar as the complication they presented is known to be associated with an increased perinatal loss. Samples of blood were obtained from the three vessels in two types of study. In Cases 2, 5, 9, 15 and 16, the patient was placed on the operating table, the abdomen was infiltrated with 1 per cent xylocaine, and the peritoneal cavity opened. With no uterine manipulation a size 18 or 20 gauge needle was inserted into the anterior uterine wall, using negative pressure throughout the manipulation. If the placenta was anteriorly located, the sample of blood from the intervillous space was collected in a 10 ml. syringe previously lubricated with oil and heparin, capped, and placed in a beaker filled with ice. Sodium pentothal (2.5 per cent) was then given intravenously, and the uterine incision performed. With delivery of the newborn four clamps were rapidly placed on the umbilical cord, two near the fetal end and the other set near the placental end. From the isolated segment samples of blood were quickly collected anaerobically from the umbilical vein and artery, respectively.

In Cases 1, 4, 6, 10, 11, 12, 13, 21 and 22, specimens of blood from the intervillous space were obtained just prior to vaginal delivery; the bladder had been catheterized, and the vertex of the fetus was on the perineum. The patients had no anesthesia. In this series a

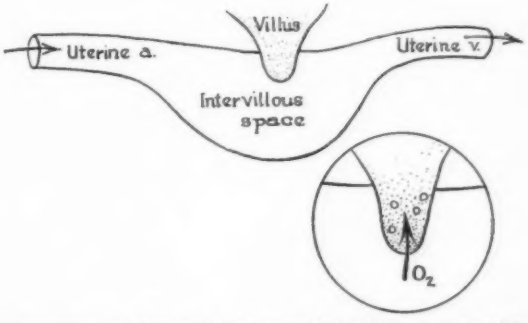

CHART 1. Graph represents relationship between intervillous space and fetal villous. Insert demonstrates that oxygen transfer occurs between intervillous space and fetal capillaries lying within the villous.

transabdominal approach was performed with a long 20 gauge needle; the puncture site was just inferior and to the right of the umbilicus. After the intervillous space sample was collected, the fetus was delivered either spontaneously or by low forceps. From an isolated segment of umbilical cord samples of blood were rapidly drawn from the umbilical vein and umbilical artery. (It was not possible to obtain samples from the artery in all cases). Fetal specimens were obtained anaerobically, capped, and placed in a beaker filled with ice. In both types of experiments the interval of time from intervillous space to cord sampling was approximately one minute.

The experimental procedure as arranged was designed to obtain intervillous blood a few seconds before sampling the umbilical artery and vein. In the estimation of the gradient this time factor is of obvious importance. Furthermore, this technic provided not only the scientific objective of the experiment but also yielded an important safety factor. In both vaginal and cesarean section groups the fetus could be delivered immediately in case of an experimental accident. It is significant to state that in the 50 experiments performed thus far only two minor fetal complications have been encountered—scratches on the fetal skin that re-

TABLE I

Data Obtained on Blood Samples Drawn from Placental Vessels—Normal Pregnancy

Date	No.	Method of Delivery	Placental Vessel	Oxygen Capacity (vols. %)	Oxygen Content (vols. %)	Per cent Saturation	Estimated pO_2 (mm. Hg)	$M_pO_2 - F_pO_2$* (mm. Hg)
10–3–56	2	Cesarean Section	Intervillous Space	14.8	8.1	54.7	27.8	17.7
			Umbilical Vein	20.2	5.1	25.0	12.5	
			Umbilical Artery	20.2	3.4	16.8	8.0	
10–10–56	4	Vaginal	Intervillous Space	15.7	8.4	53.5	27.8	15.6
			Umbilical Vein	20.6	5.1	24.7	12.2	
10–17–56	5	Cesarean Section	Intervillous Space	11.2	5.0	44.6	24.0	18.3
			Umbilical Vein	18.4	1.8	9.7	6.5	
			Umbilical Artery	18.4	1.1	5.9	5.0	
10–24–56	6	Vaginal	Intervillous Space	16.0	8.3	51.8	26.5	14.3
			Umbilical Vein	21.9	5.2	23.7	12.2	
1–8–57	10	Vaginal	Intervillous Space	15.8	15.3	96.8	72.0	33.0
			Umbilical Vein	20.7	17.4	84.0	39.0	
1–23–57	12	Vaginal	Intervillous Space	22.4	19.2	85.7	48.0	27.0
			Umbilical Vein	21.2	10.0	47.1	21.0	
1–31–57	13	Vaginal	Intervillous Space	14.1	10.8	76.5	40.1	15.1
			Umbilical Vein	18.1	10.9	60.3	25.0	
3–7–57	21	Vaginal	Intervillous Space	15.7	10.6	67.4	34.0	20.4
			Umbilical Vein	19.3	6.6	34.1	16.0	
			Umbilical Artery	19.3	3.9	20.2	11.0	
Average								20.1

* M_pO_2 = partial pressure of oxygen in maternal circulation; F_pO_2 = partial pressure of oxygen in fetal circulation.

quired no immediate or subsequent therapy. Both of these occurred in the very early part of the investigation and by instituting proper precautions no subsequent untoward happenings have appeared. Finally, in none of the parturients studied has there been any evidence of an accident such as premature separation of the placenta or damage to any placental vessel.

Shortly after the experiment the oxygen content of each sample was determined in duplicate on 0.5 ml. or 0.2 ml. specimens by the method of VanSlyke and Neill (2). The oxygen capacity was determined by the same technic as described in the previous paper of this series (3). The percentage saturation of the blood was calculated by dividing oxygen capacity (expressed in volumes per cent) into oxygen content (volumes per cent) and multiplying by 100, that is, (oxygen content/oxygen capacity) $\times$ (100) = per cent saturation. Then, the saturation was referred to an oxygen dissociation curve (4), and the partial pressure of oxygen (pO_2) in each sample was estimated. Gradients were estimated in the manner employed by Barron and Alexander (5) except that in the present study only one maternal channel was necessary for the calculation.

C. RESULTS

I. *Normal Pregnancy* (Table I): This group consists of eight patients who were considered clinically normal. Two were delivered by cesarean section (not in labor), and five via the vaginal route. The duration of pregnancy at the time of the experiment varied from 38 to 42 weeks.

(a) Intervillous Space—The oxygen content ranged from 5.0 vols. per cent to 19.2 vols per cent. Oxygen saturation varied from 44.6 to 96.8 per cent; with an average value of 66.3 per cent. The partial pressure of oxygen averaged 37.5 mm. Hg, with figures ranging from 24.0 to 72.0 mm. Hg.

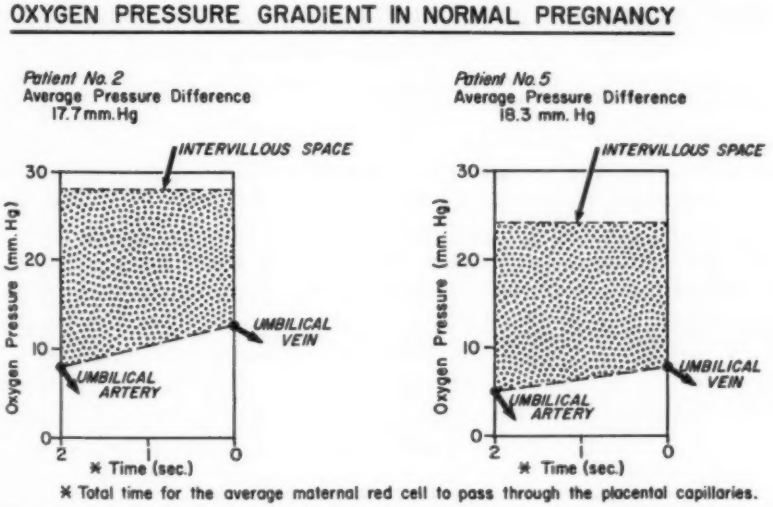

FIG. 1. A graph illustrating the estimated changes in oxygen pressure in the fetal and maternal bloods as they pass through the placental capillaries. NORMAL PREGNANCY.

(b) Umbilical Vessels—The oxygen content and percentage saturations show much variation, and this result agrees with data obtained in previous studies (6). The average pO_2 in the umbilical vein was 18.0 mm. Hg; this figure corresponds to a percentage saturation of approximately 40 per cent.

(c) Difference in pO_2 between Maternal and Fetal Circulations—Only in Cases 2, 5 and 21 is one able to calculate the oxygen "gradient" (Fig. 1). It was found to be 17.7, 18.3 and 20.4 mm. Hg, respectively. If one were to use only data pertaining to the intervillous space and umbilical vein, the average figure is 19.5 mm. Hg. Hence, it would seem that no major error is introduced by including all of the cases, whereby the difference in pressure averages 20.1 mm. Hg.

II. *Abnormal Pregnancy* (Table II): This group consists of six patients with complications suspected of yielding a reduced transfer of oxygen and one in which the mother was considered normal, but the infant was delivered and had an extremely difficult neonatal period. Three of the experiments were

TABLE II

Data Obtained on Blood Samples Drawn from Placental Vessels—Abnormal Pregnancy

Date	No.	Complication	Method of Delivery	Placental Vessel	Oxygen Capacity vols. %	Oxygen Content vols. %	Per cent Saturation	Estimated pO_2 mm. Hg	$M_pO_2 - F_pO_2$ mm. Hg
9–28–56	1	Pre-eclampsia	Vaginal	Intervillous Space	16.8	5.0	29.7	18.5	9.4
				Umbilical Vein	23.9	4.1	17.1	10.0	
				Umbilical Artery	23.9	2.1	8.7	7.0	
12–18–56	9	Pre-eclampsia	Cesarean Section	Intervillous Space	16.7	3.6	21.5	15.8	0.8
				Umbilical Vein	19.4	6.2	31.9	15.0	
1–9–57	11	Abruptio Placentae	Vaginal	Intervillous Space	15.6	3.4	21.7	15.8	1.9
				Umbilical Vein	18.2	5.1	28.0	13.9	
2–6–57	15	Spinal Hypotension	Cesarean Section	Intervillous Space	11.5	1.2	10.4	10.0	3.8
				Umbilical Vein	20.3	2.1	10.3	8.0	
				Umbilical Artery	20.3	0.9	4.4	4.2	
2–8–57	16	None	Cesarean Section	Intervillous Space	17.4	5.1	29.3	18.5	−1.7
				Umbilical Vein	20.1	9.4	46.7	20.2	
3–12–57	22	Bleeding, ? Etiology Fetal Heart Irregular and 100	Vaginal	Intervillous Space	16.8	3.9	23.2	16.2	6.9
				Umbilical Vein	23.2	5.1	21.9	11.8	
				Umbilical Artery	23.2	1.9	8.1	6.8	
Average									3.5

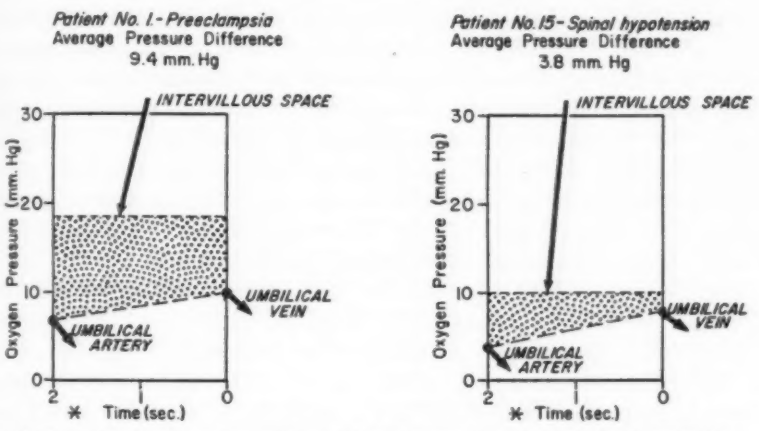

FIG. 2. A graph illustrating the estimated changes in oxygen pressure in the fetal and maternal bloods as they pass through the placental capillaries. ABNORMAL PREGNANCY.

performed at cesarean section and three at the time of vaginal delivery. The duration of pregnancy varied from 32 to 40 weeks.

(a) Intervillous Space—The oxygen content ranged from 1.2 vols. per cent to 5.1 vols. per cent (normal, 5.0–19.2 vols. per cent). Percentage saturation varied from 10.4 to 29.7 per cent (normal, 44.6–96.8 per cent), with an average value of 22.6 per cent (normal, 66.3 per cent). The pO_2 averaged 15.8 mm. Hg (normal, 37.5 mm. Hg), with figures ranging from 10.0 to 18.5 mm. Hg (normal 24.0–72.0 mm. Hg).

(b) Umbilical Vessels—As in the "normal" series there was much variation in content and saturation. It is important to note that the average saturation of the umbilical vein was 26.0 per cent and the corresponding pO_2, 13.1 mm. Hg—results very similar to those obtained in the "normal" series.

(c) Difference in Partial Pressure of Oxygen between Maternal and Fetal Circulations—In Cases 1, 15 and 22, samples of blood were obtained from all of the placental vessels; the "gradients" were 9.4 mm. Hg, 3.8 mm. Hg and 6.9 mm. Hg, respectively, with an average value of 6.7 mm. Hg (Fig. 2). If only the intervillous space and umbilical vein be considered, the difference between the two vessels averages 2.9 mm. Hg. Hence, I have included all cases, and the average figure is 3.5 mm. Hg (normal, 20.1 mm. Hg).

D. DISCUSSION

The experiments described here were carried out in an effort to obtain information about conditions in the human that would permit an assessment of

the limitations put upon the transfer of oxygen by the hemochorial, or three layered placenta. The oxygen pressure, as estimated by relating the percentage saturation of the hemoglobin in a given sample to an appropriate dissociation curve, was used to express the force driving the oxygen across the placental barrier, as if that barrier were comparable to the pulmonary epithelium. The results would indicate that the mean difference in the partial pressure of oxygen between the maternal and fetal circulations in the human is approximately 20 mm. Hg. This information would appear to be of significance in that it demonstrates a mechanism by which the mother provides oxygen to the fetus *in utero*. This has been postulated of course for a long time, but the concept has never before been quantitatively assessed in the human.

From the comparative physiological standpoint the data may also be of interest (Table III). In species with a syndesmochorial (*five* layer) placenta, sheep and goat, the gradient was found to be about 40 mm. Hg (5, 7, 8). In the rabbit which possesses a *one* layered placenta in late gestation (9) the mean difference in pressure between the two circulations is about 10 mm. Hg (10). As stated previously, the data obtained in the human's *three* layered placenta is approximately 20 mm. Hg. It is tempting to associate these differences in pressure with the number of tissue layers that separate the maternal and fetal bloods in these four forms, but further measurements of the physical characteristics of each placental type will be necessary before any conclusions can be drawn as to the particular feature or combination of features of the tissue barrier responsible for the difference in the pressure gradient.

In "abnormal" pregnancy the gradient was found to be below 10 mm. Hg. The differences between normal and abnormal gradients are outside the error of the method. Hence, this finding is interpreted as evidence that the net transfer of oxygen was reduced in these cases. If one considers oxygen necessary for the fetus, then the data bear out clinical experience, for such entities as fulminating preeclampsia and abruptio placentae are generally considered to be associated with an increased perinatal loss, secondary to hypoxia. The so-called "normal" parturient who yielded a severely distressed infant (unexplained etiology) perhaps offers us a clue as to why many fetal deaths—before, during, and after labor—are unexplained. Also, it may be a

TABLE III

Relationship between Species, Number of Tissue Layers Separating Maternal and Fetal Circulations, and Mean Oxygen Pressure Gradient

Species	Number of Layers	Mean Pressure Gradient (mm. Hg)
Goat	5	40
Sheep	5	40
Human	3	20
Rabbit	1	10

clue as to the possible etiological factor responsible for subsequent mental retardation in an infant born under ideal obstetrical circumstances.

There have appeared in recent literature many reports dealing with the "oxygen status" of the newborn. The data obtained and resultant statements have been based solely on umbilical vessel determinations. The results obtained in this series of experiments would seem to suggest that such data are of limited value, for in the observations here reported no significant difference was found in the oxygen saturation and pO_2 in umbilical vessel samples from abnormal or normal pregnancy. Also there is no consistent correlation between the oxygen levels in the umbilical circulation and the gradient. This is important for, other circumstances being equal, the oxygen supply or rate at which O_2 reaches the fetal blood will be proportional to the gradient. Although many factors play a part in providing an adequate supply of oxygen to the fetus *in utero*, the gradient may be the best index we have at the present time for this purpose. However, any intelligent attack on this problem will necessitate evaluation of other factors. These will form the substance of subsequent reports.

E. SUMMARY

Estimates have been made of the difference in the oxygen pressures of fetal and maternal bloods as they pass through the placenta. Data from eight normal parturients were analyzed; the pressure difference was approximately 20 mm. Hg. In clinically abnormal pregnancy, the pressure difference was only 3.5 mm. Hg. In these cases, therefore, the net transfer of oxygen was apparently reduced. The major difference demonstrated by the data seems to be that the maternal blood was more unsaturated in abnormal pregnancy. The data on fetal bloods in normal and abnormal pregnancies were indistinguishable so that the difference in the gradients between the two groups was accounted for by the low partial pressure of oxygen in intervillous blood.

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ABSTRACTS OF PAPERS PRESENTED BEFORE THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

April 8, 1957

NEW EVIDENCE FOR HYPOTHALAMIC CONTROL OF THE ANTERIOR PITUITARY[1]

JAMES W. WOODS[2]

Considerable indirect physiological evidence exists which strongly suggests a functional counterpart of the anatomical fact that a portal system of blood vessels links the pars distalis of the hypophysis to the brain. So far no direct measurements of anterior hypophysial secretion have been made during electrical or physiological stimulation of the cephalic end of this hypothetical pathway. If the pathway has the supposed functional significance, the rate of secretion by the pars distalis should be modifiable by stimulation of the hypothalamus.

Several investigations have demonstrated that electrical or physiological stimulation of the central nervous system can result in changes in pituitary target organ activity. The measurements, however, have been far 'downstream' from pituitary secretion. Such measurements have included ovulation as an index of gonadotrophic secretion and numbers of circulating white cells or blood or urinary steroids as indices of adrenal cortical secretion.

By making use of daily *in vivo* estimation of the rate of disappearance of I^{131} from the thyroid glands of rabbits, dogs, monkeys, or cats, it is possible to construct curves which will rapidly and quantitatively depict changes in thyrotrophic (TSH) secretion. We have made use of this technique to demonstrate that electrical stimulation of a fairly discreet area in the hypothalamus of the rabbit can increase the rate of secretion of TSH.

The experiments were carried out in the following manner: The rabbits were subjected to a surgical procedure in which electrodes were fixed in position and connected subcutaneously by fine wire leads to a small coil of wire fixed beneath the skin of the back. After recovery from the operation, they were injected with tracer doses of radioactive iodine. Two or three days after injection, twice daily measurements of radioactivity at the skin overlying the thyroid gland were started. After a "basal" rate of disappearance of radioactivity was established, stimulation of the hypothalamus was carried out for a few hours or days. The estimations of neck radioactivity were continued during the period of stimulation and for several days subsequently to re-establish the basal rate of release. Concurrent estimation of thyroid hormone in blood was carried out by chemical and by tracer methods.

The data show that in rabbits with electrodes in the anterior one third of the hypothalamus electrical stimulation results in accelerated disappearance of labelled thyroid hormone from the gland and the appearance of very large amounts of labelled and unlabelled hormone in the peripheral blood.

In some rabbits it was found impossible to elicit the response of increased TSH release in the presence of functioning adrenal cortical tissue. In these and other rabbits, the response could be unveiled by partial "chemical hypophysectomy". That is, by injecting cortisone in amounts assumed to block the natural secretion of adrenocorticotrophic hormone (ACTH).

The exact regions of the hypothalamus which must be stimulated in order to produce this response in intact or in adrenalectomized rabbits are as yet unknown. Preliminary anatomical studies suggest that electrode sites which when electrically stimulated produce the response

[1] A report of experiments carried out at the University of London under the direction of Professor G. W. Harris, F.R.S.

[2] Department of Physiology, The Johns Hopkins University School of Medicine.

only after adrenalectomy, are near but probably posterior to those which will produce the same response in the intact animal.

Direct neural influences upon the thyroid gland were ruled out by repeating the experiments after transplantation of the thyroid. Adrenal medullary factors have been partially excluded by repeating the experiments in partially sympathectomized rabbits. The crucial experiment of severing the tissue connection between stimulating electrodes and pars distalis has not been done.

CASE REPORT: SURGICAL TREATMENT OF RUPTURED ANEURYSM OF THE SINUS OF VALSALVA INTO RIGHT VENTRICLE AND ATRIUM

HENRY T. BAHNSON

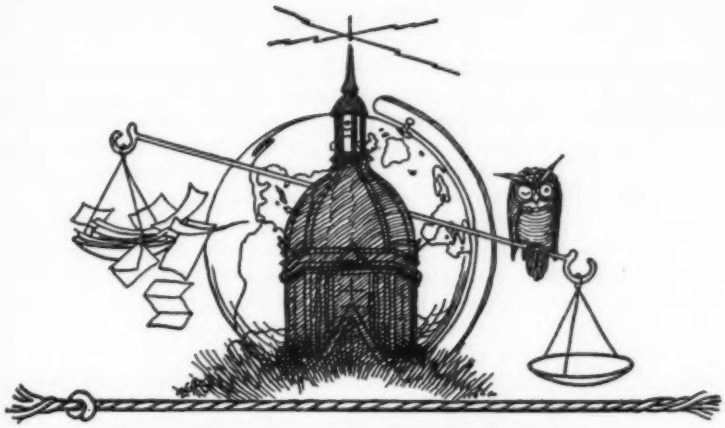
A 33 year-old mother of three had been normal and examined on a number of occasions without abnormality found. Four days postpartum there was a sudden, severe precordial discomfort and an interscapular ache with weakness, sweating, palpitation, and a comment by the nurses that the bed was rocking. An active precordium and a loud murmur were noted by her physician. She developed dyspnea and swelling of the abdomen and legs. Studies showed a generalized cardiomegaly; phonocardiogram showed the continuous murmur low along the left sternal border; and cardiac catheterization showed a large step-up of oxygen content of blood in the right atrium and ventricle. At operation on June 7, 1956, by digital exploration, a jet could be felt entering the right auricle at the valve ring. A bubble oxygenator was used during 20 minutes of cardiopulmonary bypass while the defect was repaired through an atriotomy. The edges of the defect were pulled together and a piece of Ivalon sponge was placed over it for buttress. Her convalescence has been satisfactory and at the present time she appears to be normal.

Discussion of the 45 autopsied cases available in literature was given in connection with etiology and treatment. The most plausible idea of pathogenesis seems to be that of Edwards who demonstrated a faulty attachment of the media of the annulus fibrosus of the aortic valve, this weakened area giving way.

PNEUMOCOCCAL PNEUMONIA TREATED WITH PENICILLIN AND ASPIRIN

ROBERT G. PETERSDORF, LEIGHTON E. CLUFF, PAUL D. HOEPRICH, FRANK THOMAS HOPKINS AND WILLIAM P. MCCANN

This paper is published in this issue of the Bulletin.



BOOK REVIEWS

Enzyme Antigen and Virus. A Study of Macromolecular Pattern in Action. By SIR MACFARLANE BURNET. 193 pp., $3.50. Cambridge University Press, New York, N. Y.

The evolution of a biological theory of cellular interrelationships which explains enzyme action, protein synthesis, antibody production, virus multiplication, the recognition of cell types and the origin of cancer is a task that only a man of Sir Macfarlane's energy would undertake. Yet here it all is wrapped up in a little hand book that may easily be put into a pocket and pulled out at convenience.

The concepts which Burnett and Fenner developed in their *Production of Antibodies* some eight years ago have now been expanded into a general theory of macromolecular pattern in action as intrinsic in the study of self and not self as the cell looks at it. To requote the original statement of Burnet and Fenner: "The basis of our account is the recognition that the same system of cells is concerned both in the disposal of effete body cells (without antibody response) and of foreign organic material (with antibody response). In order to allow this differentiation of function, expandable body cells carry selfmarker components which allow recognition of their 'self' character." Subsequent to this original statement, a remarkable series of studies, particularly by Medawar, has shown that cells from foreign hosts when inoculated into embryos induce recognition by the subsequent adult chicken of these cells as "self", so that foreign grafts from the first host now "take" in the new host. These studies in turn are of course extensions of the freemartin studies by Owen and others, but Dr. Burnet introduces a provocative theory to stimulate new work.

Sir Macfarlane has devoted considerable discussion to H. N. Green's (not to be confused with H. S. N. Greene of Yale) theory of the origin of cancer. This theory proposes that cancer cells are ones in which a loss of self markers is a central feature in the development of malignancy. The cell which has lost the self marker is a somatic mutant it is true, but it is assumed that the loss of the self marker has allowed the cell to proliferate.

There is a chapter on virus multiplication, in which Burnet now considers the analogy between animal viruses and bacteriophage so superficial and so little pertinent to the study of animal viruses that it is sidestepped, and major stress is laid on the RNA replicating mechanism of influenza virus.

The last chapter is a statement of belief. To this reader it was the least necessary, for Dr. Burnet seems to feel that both the scientific work and the theories behind it need public

justification as means to practical ends. This is of course sticky ground and Dr. Burnet, who has made so many very basic and important yet not immediately "practical" discoveries himself, deftly switches onto the question of the role of biological generalizations. He presents ample proof that there is plenty of room for a biologist to think and theorize, for instance about the "specific complementary pattern" which may be the means of information transfer from cell to cell.

FREDERIK B. BANG

Glaucoma. Transactions of the First Conference, December 5, 6, and 7, 1955. Edited by FRANK W. NEWELL. 251 pp., $4.50. The Josiah Macy, Jr. Foundation, New York, N. Y.

In the past 15 years The Josiah Macy, Jr. Foundation has sponsored about 25 multiprofessional scientific conferences aimed primarily at the promotion of exchanges of ideas and stimulation of creativity among the participants. These conferences constitute a multidiscipline approach to some urgent problems in the field of health.

This book comprises the lively discussions of the 19 participants at the first Macy Foundation Conference which took place in 1955. In several respects this meeting is a corollary to the International Symposium on Primary Glaucoma at Ste. Marguerite in 1954.

The book is divided into three chapters. In the beginning of the first one titled "A Review of Angle Closure Glaucoma" the reader might get the impression that the conference is doomed because of unrelenting argumentation about terminology but this fear is soon dispelled as the members engage in a discussion concerning the "pupillary block" versus the "angle block" mechanism as the primary cause for acute congestive or angle-block glaucoma. Most of them agree that a relative pupillary block is the primary basic mechanism and that the increased intraocular pressure of angle closure glaucoma is the result of the iris root blocking the outflow of aqueous through the trabeculae. The first chapter is the most *clinical* of the three including considerable discussion of gonioscopy and peripheral iridectomy in closed angle glaucoma as well as basic physiological-pathology.

The second and shortest chapter deals with the central control of intraocular pressure. It also touches on the question of innervation of the trabeculae. The laborious animal experiments outlined are ingeniously conceived and beautifully executed with the aid of the most modern electronic equipment available. Whether the results have any bearing on the problem of glaucoma is doubtful.

The last chapter "Physiologic and Pharmacologic Factors Influencing the Resistance to Aqueous Outflow" includes the most stimulating and enlightening discussions. One of the most fundamental problems of aqueous dynamics is analyzed from a variety of approaches. Does the resistance to aqueous outflow in the normal or glaucomatous eye reside in the trabecular meshwork or does it reside peripheral to Schlemm's canal somewhere along the outflow drainage channels? Here again, as at Ste. Marguerite, this question remains unanswered. Some evidence is cited that in human eyes the resistance may be peripheral whereas in certain animals the resistance may reside in the trabeculae. Excellent reviews of studies dealing with the effect of hyaluronidase on the resistance to aqueous outflow are presented in detail. There is also considerable discussion of homeostatic and consensual reflexes involved in the control of intraocular pressure. An extensive bibliography, appendix and index are included.

Unfortunately, the discussions, particularly in the first chapter, rather frequently wander off the main tracks. They range from hypnosis and the use of the metronome to scleral buckling operations in retinal detachment. What may be a little more worrisome to the critical reader is the tendency by some participants to advance important interpretations based only on individual cases or very few observations. These annoyances, however, are

minor considering the volume of valuable information the reader will derive from this book. By and large the discussions are kept on a plane which can easily be followed by any serious student of ophthalmology. Though this book will probably appeal more to those particularly interested in glaucoma research, this reviewer feels that it should be of particular value to trainees in ophthalmology.

Finally, conference-minded ophthalmologists might well take notice that this was a major ocular symposium in which some of the most active participants came from nonophthalmological disciplines. More of such conferences will undoubtedly serve as great stimuli to all concerned, particularly, if moderated by men as keen and with a knowledge as broad in scope as that of Dr. Frank Fremont-Smith, medical director of the Macy Foundation.

HOWARD H. STONE

The Structure and Function of Skin. By WILLIAM MONTAGNA. 356 pp., $8.80. Academic Press, Inc., New York, N. Y.

Approximately ten years ago the publications of William Montagna attracted the attention of dermatologists engaged in investigative work. Young and comparatively unknown at the time, Montagna exhibited so much talent and enthusiasm that it was obvious he was destined for greatness. He classified himself as an anatomist with an interest primarily in the structure of the skin. However, it was soon discovered that he was not only an anatomist but also a physiologist, biologist, chemist, poet, linguist and gift to dermatology in one package. He was quickly and thoroughly integrated into dermatology through many collaborative studies as well as by his teaching activities in the Academy of Dermatology.

The Structure and Function of Skin is a scholarly presentation of the embryology, histology and anatomy of the skin in terms of function as well as structure. A critical selection of the important contributions of the past has been combined with numerous contemporary and personal observations and discoveries to present an authoritative thesis in terms of modern technics and values. The author interprets gross alterations, cytologic changes, results of histochemical technics and electron microscopy with such clarity and conviction as to leave no doubt concerning his great versatility and broad scope as an investigator.

This book is highly recommended.

MAURICE SULLIVAN

Physiologic Principles of Surgery. Edited by LEO M. ZIMMERMAN AND RACHMIEL LEVINE. 988 pp., $15.00 W. B. Saunders Co., Philadelphia, Pa.

This large book well attains the authors objective of describing the known physiological abnormalities in surgical disorders. The textbook is a unique one in that regard, and will be a valuable reference source for physiological abnormalities in surgical diseases. All chapters contain an extensive bibliography. There is little information concerning diagnosis or treatment; so for clinical problems the book is of value mainly as a reference source for the principles underlying treatment.

A vast amount of experimental and clinical data have been evaluated in each chapter; so readers will find the book contains information previously available only by extensive review of the medical literature.

FRANK SPENCER

BOOKS RECEIVED FOR REVIEW

The Adrenal Cortex. By I. CHESTER JONES. 316 pp., $7.00. Cambridge University Press, New York, N. Y.

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